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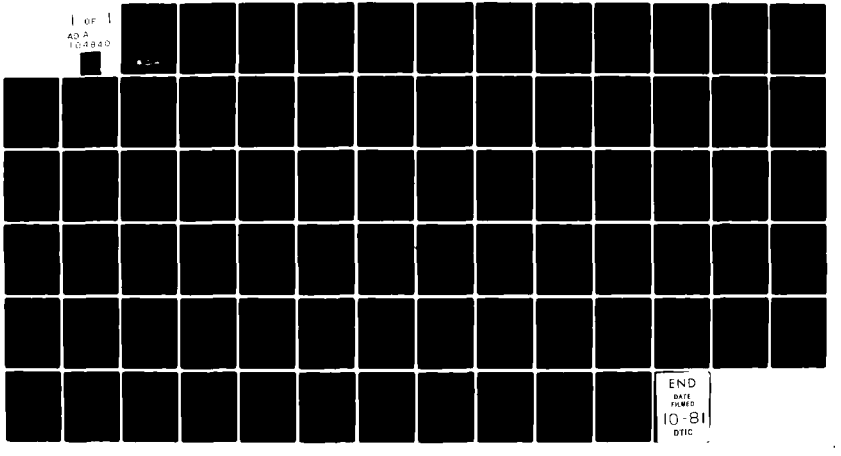

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INSTITUTE REPORT NO. 87

INVESTIGATION OF POSSIBLE ANITIVITAMIN B-6 PROPERTIES IN IRRADIATION STERILIZED CHICKEN

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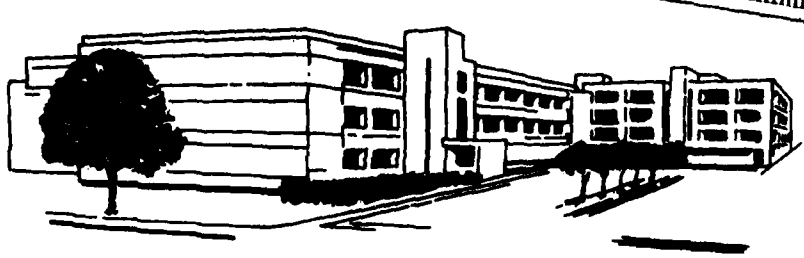
JUNE 1981



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Investigation of Possible Antivitamin B-6 Properties
in Irradiation Sterilized Chicken--McGowen et al

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

Jean M. Marshall Jr 13 Aug 81
(Signature and date)

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The purpose of the study was to determine whether irradiation (gamma or electron) or thermal processing of chicken produces factors which are antagonistic to vitamin B-6 in the diet of rats. (These methods of preservation all result in lowered vitamin B-6 contents relative to frozen chicken.) Male and female rats (156 each) were made vitamin B-6 deficient by feeding a semi-purified diet devoid of vitamin B-6. They were then repleted with various test diets containing chicken which had been preserved by one of four methods: frozen, thermally processed, electron

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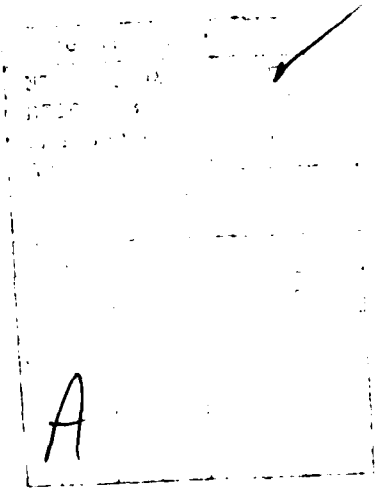
or gamma irradiated. All repletion diets were supplemented to contain carefully controlled (marginal or high) levels of vitamin B-6. Recovery rates were monitored by growth (weight gain) and measurements of vitamin B-6-dependent blood enzymes (plasma and red cell aspartate aminotransferase and alanine aminotransferase). No differences were observed in weight gain among the chicken-fed groups. The enzyme responses of rats fed frozen, thermally processed or electron irradiated chicken were similar. Responses of some of the enzymatic parameters were slightly delayed in the groups fed gamma irradiated chicken at the marginal vitamin level. No consistent differences were observed between any of the high vitamin groups. If antivitamin B-6 factor is present in gamma irradiated chicken, it is minimal, is detectable only under conditions of marginal vitamin B-6 status, and is overcome by added dietary pyridoxine.

ABSTRACT

The purpose of the study was to determine whether irradiation (gamma or electron) or thermal processing of chicken produces factors which are antagonistic to vitamin B-6 in the diet of rats. (These methods of preservation all result in lowered vitamin B-6 contents relative to frozen chicken.) Male and female rats (156 each) were made vitamin B-6 deficient by feeding a semi-purified diet devoid of vitamin B-6. They were then repleted with various test diets containing chicken which had been preserved by one of four methods: frozen, thermally processed, electron or gamma irradiated. All repletion diets were supplemented to contain carefully controlled (marginal or high) levels of vitamin B-6. Recovery rates were monitored by growth (weight gain) and measurements of vitamin B-6-dependent blood enzymes (plasma and red cell aspartate aminotransferase and alanine aminotransferase). No differences were observed in weight gain among the chicken-fed groups. The enzyme responses of rats fed frozen, thermally processed or electron irradiated chicken were similar. Responses of some of the enzymatic parameters were slightly delayed in the groups fed gamma irradiated chicken at the marginal vitamin level. No consistent differences were observed between any of the high vitamin groups. If an antivitamin B-6 factor is present in gamma irradiated chicken, it is minimal, is detectable only under conditions of marginal vitamin B-6 status, and is overcome by added dietary pyridoxine.



FOREWORD

Recent U.S. Army protocols for the wholesomeness testing of radappertized meats and poultry (1,2,) included antivitamin studies for thiamin and vitamin B-6. These studies were designed in response to questions raised by the Food and Drug Administration (FDA) in 1968, after review of data in the readappertized ham petition. This foreword provides a brief historical background that led to the requirement for the antivitamin studies in the irradiated food protocols.

In 1953, the U.S. Army was assigned the task of determining the wholesomeness of foods preserved by radappertization (sterilization by ionizing radiation) and of developing the process. Protocols were designed with the assistance of scientists from industry, universities, and FDA. These studies were essentially completed by about 1964. In general, the foods under study were canned and irradiated at ambient temperature at two dose levels (2.79 and 5.58 mrad) with spent fuel rods. Radappertized foods were stored at room temperature and the control canned foods were stored frozen.

Bacon was one of the most promising foods preserved by radappertization. In 1963, a petition for cobalt-60 radappertized bacon was approved by FDA (3). Subsequently, approval was obtained for irradiation with 5-10 MeV electrons, 5 MeV x-rays, and Cesium-137. With the approval of the bacon petition, a petition for ham was submitted. This petition was based on bacon and pork data. Expert opinion was in agreement; since ham was intermediate in degree of processing to bacon and pork, it did not need to be tested. After review of these data in 1968, FDA concluded, in part, "Our evaluation of the raw data led us to believe that there were suggestions of adverse effects and that, therefore, the safety of these irradiated meats had not been established" (4p96).

Subsequently, the U.S. Army withdrew the ham petition and FDA rescinded its previous approval of radappertized bacon (4pp123-124). Although some of the suggested adverse effects reported by FDA were a matter of opinion and interpretation, it is not the intent or purpose of this foreword to rebut them. However, it should be recognized that the pioneering U.S. Army irradiated food program was initiated before the Food Additives Amendment of 1958 and its later rigorous interpretation and enforcement (4pp127-132).

Pertinent to the present issue, an adverse effect was observed "apparent production of antinutrient factors" (4p104). This conclusion was based primarily on incomplete data in the progress reports of Brins' group (5-7) in which blood transketolase and transaminase activities were reduced in rats fed radappertized pork, unsupplemented with thiamin or pyridoxine, respectively, and were not restored to normal upon repletion with the respective vitamin (5,6). If the pork

diets were supplemented initially with the vitamins, the enzyme activities were not affected by the irradiated pork (5,7). Although the studies might be questioned, this foreword will not discuss the merits of the data or their interpretation by FDA; however, it would appear that when the wholesomeness of radappertized foods is under consideration, the question, i.e., production of antinutrient factors, raised by FDA must be resolved to the satisfaction of all concerned.

Some persons question the value of the "old" wholesomeness studies. Irradiation technology has not only changed and improved, but also the requirements for additive testing have become more stringent. Since the present day radappertized products are superior to those first-generation products, it could be assumed that the earlier products presented the worst possible case. Also, if any irradiation-induced toxicity had been present, it would have manifested itself more prominently. In spite of this, when adverse effects were found, subsequent investigations disclosed that these were not caused by irradiation but resulted from other complicating factors. One such problem, which generally has been given inadequate consideration, was that radappertized foods (very highly processed and stored at ambient temperature) have been (unfairly) compared to unprocessed frozen controls. Furthermore, the wholesomeness studies have attempted to prove a negative -- virtually an impossible task.

Investigators (8,9) from Letterman Army Institute of Research have already published two reports on thiamin and erythrocyte transketolase activity in which they used irradiated beef (8) and irradiated chicken (9). The following report describes the effects of dietary pyridoxine levels and radappertized chicken on blood transaminase activities in rats.

NICHOLAS RAICA, JR.
Consultant
U.S. Army Medical Research
and Development Command

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PREFACE

The experimental portions of the study covered in this report were conducted during the period 1 November 1979-1 June 1980. All raw data are being stored at Letterman Army Institute of Research. Anyone wishing to examine the raw data or to obtain copies of tables containing individual values may do so by contacting:

Commander
Letterman Army Institute of Research
ATTN: SGRD-ULZ
Presidio of San Francisco, California 94129

In addition to personnel listed on page xii, the authors gratefully acknowledge the assistance of COL Ronald Johnson (MOBDES) who helped interpret the data and prepare the report, and Ann Wilkinson and Carol Allen, typists. We particularly thank Lottie Applewhite, LAIR Technical Editor, for her editorial assistance and for detecting several potentially serious omissions.

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DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

8 January 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 79002 the following inspections were made:

20 December 1979
2 January 1980
18 January 1980
8 February 1980
29 February 1980
12 March 1980
19 March 1980

Findings were reported to the Study Director and laboratory management on 11 February 1980. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the May 1980 report to management and the Study Director.

JOHN L. SZUREK
MAJ, MS
Quality Assurance Officer

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Fort Detrick, MD

Signatures of Principal Scientists
Involved in the Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretations to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies outlined by the Food and Drug Administration.

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Study Director

INTRODUCTION

The testing of control and irradiated meats for antimetabolite activity against vitamins B-1 and B-6 is a requirement of the protocol entitled "Animal Feeding Protocol for Irradiation Sterilized Test Foods" originated by the Office for Wholesomeness of Irradiated Foods, U.S. Army Medical Research and Development Command (USAMRDC), dated 21 October 1975.

The purpose of the study reported here was to determine whether irradiation (gamma or electron) or thermal processing of chicken produces factors which are antagonistic to vitamin B-6 in the diet of rats.

Background and Experimental Design

The present study is similar to another antivitamin study which was conducted at this institute (1). The same lot of chicken was used and many of the standard operating procedures were identical. Some of the experimental details common to both studies have already been described (1) and will be cited rather than repeated in this report.

The protocol for the antivitamin B-6 study specified that rats were to be made deficient in vitamin B-6 (according to a pre-set weight gain criterion). They were then to be repleted with various chicken-containing or semi-purified diets which had identical (high or marginal) vitamin B-6 contents and the recovery rates were monitored. A decreased recovery rate in animals fed irradiated meat relative to those fed control meat could indicate the presence of an antivitamin B-6 substance. Such a substance could arise (but not necessarily) from radiolysis of endogenous vitamin B-6.

Resumption of weight gain was the obvious indicator of recovery. The other parameters specified by the protocol were blood transaminases which require pyridoxal phosphate for activity: serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and erythrocyte AST. These enzymes are decreased in vitamin B-6-deficient rats and humans (2,3). Transaminase activities are much lower in

serum than in erythrocytes and the serum enzymes are not considered reliable indicators of vitamin B-6 status, at least in the human (3,4). In fact, serum AST measurements are commonly used to detect such pathological conditions as myocardial infarction rather than to assess nutritional status. Erythrocyte AST measurements and, in particular, in vitro stimulation by pyridoxal phosphate are considered to be the most useful methods for assessing vitamin B-6 status (3,4). (Erythrocyte ALT has not been a common measurement because of technical difficulty of assaying the low activity in the presence of hemoglobin in hemolysates.) The "activity coefficient" ratio of stimulated to unstimulated activity is presumably indicative of the proportion of enzyme not saturated with cofactor.

The protocol specified that the meat diets contain 35% test meat (on a dry weight basis). Furthermore, it specified that each meat be tested at two levels of vitamin intake, a marginal level and a high level. The high vitamin diets were included to determine whether or not any antivitamin B-6 substance (if detected) could be overcome by additional vitamin.

All test meats originated from one lot of chicken which had been heated to inactivate enzymes. This lot was divided into four parts and each was further prepared for storage by one of the following treatments: frozen (control); canned (thermally processed); gamma (cobalt) irradiated; electron irradiated. The last three treatments produce shelf-stable products and are known to cause decreased vitamin content. Finally, the protocol specified the inclusion of groups fed dry semipurified diets without chicken.

To conduct a successful and scientifically valid antivitamin B-6 study according to the guidelines of the protocol, we found it necessary to solve several problems before undertaking the study. The vitamin B-6 microbiological assay posed particularly challenging problems which will be described later in the Discussion. Another problem was the lack of a reliable standardized erythrocyte transaminase assay which would be suitable for large numbers of samples. Although the serum assays had been well established (in fact, commercial kits are available), erythrocyte transaminase assays left much to be desired (3). To remedy this situation, an autoanalyzer procedure was developed to assay erythrocyte AST in washed red cells and a Gemsaec fast analyzer method for whole blood AST was developed. Since we could not know in advance which method would prove more valuable, both assays were done on all samples. Activity coefficients on the plasma enzymes were also determined, although this was not specified by the protocol.

A third problem was the fact that the protocol specified marginal repletion levels to be 8.0 mg pyridoxine/kg diet, but we suspected that this level might not be low enough to provide a sensitive test system. Some reports have suggested that the B-6 requirement

of the rat is lower than 8 mg/kg (5,6). Lastly, we felt it essential to establish that repeated bleeding of rats would not cause elevated serum transaminases due to muscle damage during cardiac puncture. To answer the two latter questions and to establish the validity of the erythrocyte AST assays, we conducted a pilot study.

Pilot Study

Twenty-four female weanling rats were made vitamin B-6 deficient as indicated in the Methods. A blood sample was taken from each for transaminase analyses and the animals were divided into 3 repletion groups of 8 each. The casein-based repletion diets contained 2.0, 4.0, and 12.0 mg pyridoxine·HCl/kg diet. A group of 6 non-deficient control rats was maintained on the 12.0 mg/kg diet throughout the pilot study. Figure 1 shows the growth curves of the four groups during the repletion phase. All three repletion groups immediately exhibited striking weight gains. After 4 weeks, there was a small but significant difference in mean weights between the lowest and highest vitamin groups. There was little difference between the 4.0 and 12.0 mg/kg groups.

The erythrocyte aspartate aminotransferase data yielded similar conclusions. Within 7 days, both enzymatic activity and activity coefficients had returned to their normal ranges except in the lowest vitamin group (Figure 2). Therefore, it appeared that in order to promote marginal recovery rates, the diets had to contain less than 4.0 mg pyridoxine/kg diet and 2.0 mg/kg appeared to be suitable. However, endogenous vitamin B-6 in the frozen control chicken (at 35% dry weight) contributed more than 2 mg/kg to the diet (Table 1). We, therefore, chose 2.5 mg pyridoxine·HCl/kg diet to be the marginal repletion level.

The final question addressed in the pilot study was whether repeated blood samples could safely be taken by cardiac puncture. We found no evidence that cardiac puncture caused elevated serum transaminases within the time frame which would interfere with our study (data not shown). Although an occasional sample did have elevated values, this occurred so seldom that we did not feel it posed a threat to the study.

METHODS

Chicken Test Meats

All test meats were supplied by the U.S. Army Natick Research and Development Command, Natick, MA. They were from a lot labelled "Lot 3" and "Lot 3A" and had been processed according to the procedure outlined in Appendix A of the protocol described in paragraph 1 of page 1. The enzyme inactivation, gamma irradiation and electron irradiation have been summarized in our previous report (1).

Animal Care

Male and female weanling rats (156 each) were purchased from Charles River Breeding Laboratories, Wilmington, MA. Each animal was identified by ear tag and individually caged in a room with a 12-hr light/dark cycle. All were given ad libitum water and a semi-purified diet containing 12.0 mg pyridoxine·HCl/kg diet. The casein-based diet was the same as described previously (1) except a commercial salt mixture was used (Rogers & Harper salt mix (7), purchased from ICN Nutritional Biochemicals, Cleveland, OH, and added as 4% of the diet).

The schedule and diet codes for the two studies are outlined in Table 2. After one week of quarantine and adaptation (Phase 1), 24 rats were selected at random to remain on diet A. All other animals were placed on diet B, which was identical to diet A, except that pyridoxine had been omitted (Phase 2). Growth was monitored throughout the study by thrice weekly weighings. Animals on diet B were considered to be deficient when the average daily weight gain was less the 1.0 g. The deficient animals were then randomly divided into 11 groups of 12 animals each. The diet A animals were also divided into 2 groups of 12 each. One group of 12 diet A rats and one group of 12 diet B rats were bled by cardiac puncture and removed from the study. The remaining 10 groups of deficient animals were placed on 10 different repletion diets (C through L) and the remaining A group was continued on diet A. The repletion period (Phase 3) lasted 4 weeks.

Diet Preparations

Values for proximate analyses, calcium, and phosphorus were reported previously (1). Vitamin B-6 assays were done by a microbiological method which utilizes Saccharomyces uvarum (formerly S. carlsbergensis) as the test organism (8).

As specified by the protocol, the meat diets were formulated to contain 35% (dry weight) chicken. The fat and protein levels of the semi-purified diets were adjusted to be similar to the meat diets, based on calculations from proximate analysis data (1). For each of the meat treatment groups (E through L), a dry premix with fat and protein omitted and containing the proper amount of pyridoxine·HCl was prepared in advance. When mixed with the corresponding meat (35% dry weight basis), the complete diets contained the specified levels of vitamin B-6 and were similar to the semi-purified diets, except that ground chicken replaced the casein, lard, and corn oil. Further details of the mixing and feeding procedures were included in the previous report (1).

Blood Sampling and Analyses

Blood samples were obtained by cardiac puncture from all rats on days 7, 14, and 28. The animals were anesthetized with penthrane gas

and samples (2.5 ml each) were collected into EDTA-containing syringes. Hematocrits were determined in duplicate on each sample by the microcapillary centrifugation method. Aliquots of each were centrifuged and the plasmas were removed (in subdued light) for AST and ALT assays (done on the same day as the blood drawing). The red cells were washed twice with saline, hemolyzed with distilled water, and stored frozen until erythrocyte AST assays were done.

AST activity in sera and whole blood hemolysates (18 X dilution of previously frozen whole blood with 0.9% saline containing 0.1% Triton X-100) was determined with a Gemsaec Clinical Analyser. The chemistry employed was a slight modification of the method recommended provisionally by the International Federation of Clinical Chemists (IFCC) (9); the principal modifications consisted of lower concentrations of substrates and lower coupled enzyme activities, lower sample to total reactiom volume ratio, and initiation of the reaction with sample rather than reagent. Serum ALT activity was determined using similar modifications of the chemistry recommended by the Scandinavian Society for Clinical Chemistry and Clinical Pathology (SSCP) (10). The details of these methods have been published in a handbook of vitamin B-6 methodology (11). Activities were determined with and without exogenous pyridoxal-5'-phosphate.

Erythrocyte AST (EC 2.6.1.1.) was measured by an adaptation of the method described in Technicon Method SE4-0010 FH4 (12) for serum analyses, based on the work of Kessler et al (13). The reagent concentrations were adjusted to permit optimization of the enzyme reactions as recommended by the IFCC (9,14). The apparatus was modified to allow the semi-automated measurement of pyridoxal phosphate-stimulated AST activity under system-controlled reaction conditions. These modifications are described elsewhere (11).

Although erythrocyte ALT measurements had not been specified in the protocol, inspection of erythrocyte AST and plasma ALT data suggested that erythrocyte ALT data might be useful. Therefore, an autoanalyzer assay was developed and the erythrocyte samples were reassayed for ALT (two months after the original AST analyses). The procedure for the measurement of erythrocyte ALT (EC 2.6.1.2.) was based on the method recommended by the SSCP (14) as described in Sigma Tech. Bulletin No. 57-UV (7-79) (15) for the analysis of serum. Reagent relationships were modified as required to adapt the procedure for use with the continuous flow analytical technique for the Technicon AutoAnalyzer II. Duplicate analyses for erythrocyte ALT activity were performed in the presence and absence of pyridoxal phosphate to derive activity coefficients.

Data Acquisition and Handling

Animals were weighed using an electronic balance interfaced with a programmable calculator. The weight data were permanently printed on

paper tape and recorded on a miniature tape cartridge. The information on the magnetic tape was then transferred to a minicomputer (Data General Eclipse C330) to be processed and released in report format. Programs for transferring and processing the data were developed locally in the Information Sciences Group.

Plasma aminotransferase data were expressed as IU/liter on the print-out associated with the Gemsaec Fast Analyzer. These data were manually transferred to code sheets and card punched. Erythrocyte aminotransferase data were recorded in digital printout form as "chart units" on paper tape from the autoanalyzer. These data, appropriate conversion factors, and hematocrit values were also manually entered onto keypunch coding sheets. Calculations of enzymatic activity in terms of IU/ml red cells were done by the computer (programming by the statistician) in conjunction with the statistical analyses.

Quality control data for both plasma and erythrocyte aminotransferase activities are summarized in Table 3.

Statistical Analysis of Aminotransferase Data

Past experience has demonstrated the effects of diet and level of vitamin B-6 to be different for the different sex groups. Hence, the measurements taken on the three sample days (7, 14, and 28) were analyzed separately for each sex to determine the main effects of the design variable (i.e., diet and level of vitamin B-6) along with their interactions on each aminotransferase and its activity coefficient.

Based on the assumptions of normality, statistical independence and the equality of subgroup variances, two-way analyses of variances were performed with a packaged computer program, BMDP Biomedical Computer Program P2V (16).

The following model was used in the two way analyses of variances:

$$y = m + a_i + b_j + ab_{ij} + e$$

where y = observed enzymatic activity

m = overall mean effect

a_i = effect due to diet group

b_j = effect due to vitamin level

ab_{ij} = effect due to diet by vitamin level interaction

e = error term

When the results of the analysis of variance gave a significant F value for diet effects, a posteriori multiple comparisons were used to test for differences among means by the Newman-Keuls procedure (17). In some of the multiple comparisons, the Newman-Keuls test was unable to find significant group differences and therefore, 95% confidence intervals were also examined. Three cases were found in which analysis of variance (ANOVA) suggested diet effects, but neither Newman-Keuls nor the confidence intervals revealed any diet differences within the vitamin levels. The variances in these cases were significantly different by Bartlett's test procedure (17). Therefore, the Kruskal-Wallis one-way analysis of variance procedure (18) was used separately on each vitamin group in these three cases to test group differences. Differences were found in two of them and these have been noted in the ANOVA summaries.

RESULTS

Vitamin B-6 Analyses

Results of vitamin B-6 analyses in the chicken meats are presented in Table 1. Frozen control chicken had the highest vitamin B-6 content. Thermally processed, electron irradiated, and gamma irradiated had 83%, 63%, and 50%, respectively, of the frozen level. Table 1 also includes the calculated amounts of vitamin B-6 contributed by the chicken in the diets as well as the amount of crystalline pyridoxine added to each to obtain the desired total (2.5 or 12.0 mg/kg). It is obvious that most of the vitamin B-6 in the marginal frozen and thermal chicken diets had originated in the meats. In contrast, approximately half of the vitamin in marginal gamma and electron diets was exogenous pyridoxine.

The vitamin B-6 contents of the test diets as determined by microbiological assays are also summarized in Table 1. Among the marginal diets, the frozen control consistently assayed slightly higher than the other groups, despite the fact that the diets had been carefully formulated to contain identical amounts. The reasons for the apparent discrepancy will be discussed later.

Animal Body Weight Responses

Growth (weight) curves for both males and females during the quarantine and depletion phases are shown in Figure 3. Non-deficient and deficient groups had similar weight changes for 10 days, after which growth slowed considerably for both males and females on the deficient diets. The criterion for deficiency of weight gains less than 1.0 gram/day was met at 33 and 42 days for females and males, respectively. Because males required longer to reach this state of deficiency, their body weights were much greater than the females at the beginning of the repletion phase.

Growth curves for the rats fed the repletion diets are presented in Figures 4-8. In each case, the growth curve for the non-deficient control group is shown to simplify the comparisons. Apparent jogs in the curves occurred after 7 and 14-day bleedings, presumably because of physiological stress on the rats. Occasional animals died following bleeding days, but we included their body weights with the data recorded prior to death.

When all groups are compared, it is obvious that rats consuming meat diets regained their weight faster than those fed the semi-purified repletion diets. This could be attributed to the fact that the rats fed meat diets ate more feed (on a dry-weight basis) than those fed the semi-purified diets (data not presented), presumably because the meat diets were more palatable.

When fed meat diets, females regained their weight and caught up with the non-deficient controls by or before the end of the repletion phase. In contrast, although all male groups exhibited marked recovery of weight, none of them reached the weight of the non-deficient control group by day 28 of repletion. (This was probably due to the larger difference between controls and deficients after the longer depletion phase.) Neither males nor females recovered completely when fed the semi-purified repletion diets.

Means for initial and final weights for the repletion phase and average daily gains calculated by week and for the total 28 days are shown in Tables 4 and 5 for males and females, respectively. Inspection of these data, as well as Figures 4-8, reveal no differences between the marginal and high vitamin groups. Thus the level of 2.5 mg pyridoxine per kg diet apparently satisfied the repletion requirement for vitamin B-6 as far as growth criteria are concerned. Furthermore, no differences appeared between any of the meat groups, although all of them supported better gains than the semi-purified repletion diets.

Erythrocyte Aspartate Aminotransferase (AST)

Analysis of variance significance levels for erythrocyte AST are presented in Table 6. Both enzymatic activity (unstimulated) and activity coefficients were sensitive to vitamin B-6 intake, as revealed by the highly significant and consistent P values in the vitamin effect column. Significant diet effects ($P < .05$) were detected for enzymatic activity in 4 out of 6 sampling days and on all days for activity coefficients. The sources of these treatment effects will be examined later.

Erythrocyte AST group means are graphically summarized in Figures 9 (males) and 10 (females). Before repletion, erythrocyte AST in the deficient males and females was 25% and 40%, respectively, of the levels in the corresponding non-deficient controls. Within 7 days,

all repletion groups were well above deficient levels and by day 14, they were at or above the levels of the non-deficient animals. For all practical purposes, one might consider all groups to have recovered within 2 weeks when compared to animals which had never been deficient. However, the high and marginal vitamin repletion groups remain clearly distinguishable through 28 days.

Activity coefficient results are summarized in Figures 11 (males) and 12 (females). At day 0, the deficient groups had mean activity coefficients of 1.94 (males) and 1.68 (females) compared to 1.06 for non-deficient animals (both sexes). By day 7, all groups were well below the deficient values, and by day 14, were at or near the non-deficient levels. In agreement with the enzymatic activity results described above, the effect of vitamin intake was obvious through 28 days: all high and low vitamin lines in Figures 11 and 12 are clearly separated. The only obvious effect of diet was the tendency for the semi-purified (marginal) group to recover more slowly.

Tables 7 and 8 give group mean values and the results of the Newman-Keuls multiple comparison tests. Some significant group differences were detected, but none was consistently observed throughout all sampling days. The semi-purified group was slowest to recover (lowest activity and highest coefficients during the first 2 weeks). The gamma irradiated groups tended to recover slower than the frozen controls, but this was statistically significant for enzymatic activity only on day 7 (males), and for activity coefficient on days 7 and 28 (both sexes). The groups fed electron irradiated chicken fared as well or better than the frozen control group.

Plasma Aspartate Aminotransferase

Analyses of variance significance levels for plasma AST are summarized in Table 9. Plasma AST was less sensitive to vitamin intake than the erythrocyte enzyme and P values were significant only on day 7. However, the activity coefficient was significantly ($P < .05$) affected by vitamin level in all cases except day 28 for males.

Graphic comparisons of plasma AST group means are shown in Figures 13 (males) and 14 (females). At day 0, the deficient animals had activities of 38% (males) and 35% (females) of their respective non-deficient controls. By day 7 of repletion, all groups were nearly back to non-deficient levels. The effect of vitamin intake (high vs. marginal) was obvious for all treatments on day 7 (males), for irradiated treatment on day 7 (females), and for frozen, thermal, and gamma treatments on day 28 (males). On other days, there was no clear-cut separation between high and low vitamin groups.

Activity coefficient group means are graphed in Figures 15 (male) and 16 (females). At day 0, the deficient animals had activity coefficients of 2.5 (males) and 2.7 (females) compared to 1.2 and 1.3

for respective non-deficient groups. By day 7, all groups had returned to non-deficient levels or nearly so. Although the marginal vitamin groups tended to have higher activation coefficients than the high vitamin groups, this was not consistent except on days 7 and 14 in the males.

Plasma AST group means and results of Newman-Keuls multiple comparison tests are summarized in Tables 10 (AST activity) and 11 (activity coefficients). Some group means were significantly different ($P < .05$) but no consistent trend was observed. The groups fed gamma irradiated chicken tended to recover slower than those receiving frozen chicken. This trend was significant only on day 7 for males and on days 14 and 28 for females (activity coefficient only). In agreement with erythrocyte AST parameters, the electron-irradiated groups were not different from the frozen control groups.

Plasma Alanine Aminotransferase

Analysis of variance significance levels for plasma ALT and activity coefficient are presented in Table 12. Both parameters were highly sensitive to vitamin intake as indicated by the low P values in the vitamin effects column. Some effects of diet were detected and these will be examined later.

Graphic summaries of plasma ALT group means are shown in Figures 17 (males) and 18 (females). At day 0, the activities in plasma of deficient animals were 28% (males) and 13% (females) of the respective control values. By day 7 of repletion, the marginal groups had increased to nearly the levels of non-deficient controls and the high vitamin groups had actually surpassed them. The high vitamin groups remained elevated throughout the 4-week observation period. Along with this dramatic and persistent overshoot of plasma ALT, the high and marginal vitamin repletion groups were clearly differentiated at all times. The magnitude of the separation was greater than had been observed with the plasma or erythrocyte AST. After day 7, there was little further improvement of the marginal vitamin groups in either sex with respect to the non-deficient controls or the high vitamin groups. The only apparent effect of diet was the tendency for the frozen (marginal) group to have higher enzymatic activity than the other marginal groups.

Plasma ALT activity coefficients are summarized graphically in Figures 19 (males) and 20 (females). Deficient males had activity coefficients of 2.0 and females 2.5 compared to 1.2 for non-deficient animals (both sexes) on day 0. By day 7, all high vitamin repletion groups had dropped to non-deficient levels or below. Although all marginal repletion groups had strikingly improved by day 7, their activity coefficients remained elevated and distinct from the high vitamin repletion groups throughout the 4-week observation period. There was no obvious effect of diet except, perhaps, the faster

recovery of the males fed frozen chicken (marginal vitamin). Although deficient females had higher activity coefficients, they recovered faster and more consistently than did the males.

Plasma ALT group means and results of Newman-Kuels comparisons are summarized in Tables 13 (enzymatic activity) and 14 (activity coefficients). Few effects of diet were detected, and whenever differences were found, the frozen group always had highest enzymatic activity and lowest coefficients. The gamma-fed males did differ significantly from the frozen group on days 7 and 14 (both parameters). However, this was not observed in the females except on day 7, and then only in enzymatic activity (Table 13).

Erythrocyte Alanine Aminotransferase

Because of the high sensitivity and reliability of plasma ALT as an indicator of vitamin B-6 intake, we felt it would be worthwhile to examine the erythrocyte enzyme. To do so required development of an analytical method. We adapted the autoanalyzer method for erythrocyte AST and improved the sensitivity to accommodate the lower ALT activity in the erythrocyte (approximately 20% of AST levels). The assays were done on the samples which had been assayed originally for erythrocyte AST. By the time the ALT assay could be done, the samples had been stored frozen (-70°C) for approximately 3 months. We have since found ALT to be stable for at least 3 months when hemolysates were stored under these conditions (data not shown).

Analysis of variance significance levels for erythrocyte ALT and activity coefficients are given in Table 15. Both parameters were highly sensitive to vitamin intake except the activity coefficient on day 28 in the females. Many significant effects of diet and diet-vitamin interactions were detected which will be examined below.

Graphic summaries of erythrocyte ALT group means are shown in Figures 21 (males) and 22 (females). At day 0, the deficient groups had 12% (males) and 20% (females) of the activity of the non-deficient controls. During the four weeks of repletion, progressive increases were observed in all groups. The high vitamin groups increased faster, but had not quite returned to non-deficient levels by 4 weeks of repletion. The only consistent effect of diet was the fact that at all time points, the semi-purified marginal group had the lowest group mean.

Erythrocyte ALT group means and results of Newman-Keuls comparisons are summarized in Tables 16 (enzymatic activity) and 17 (activity coefficients). Among the marginal groups, the semi-purified consistently had the lowest activity and the frozen group the highest (except for the unusually high electron mean on day 7 in the males). The marginal gamma group means were lower than the marginal frozen, but were significantly so only on days 14 and 28 (both sexes). The

high gamma group was also significantly lower on day 7 (females) and day 28 (both sexes). Electron irradiated was significantly lower than the frozen control in 4 out of 6 comparisons of the marginal vitamin level and slightly, but significantly lower in 3 of the 6 comparisons at the high vitamin level.

Activity coefficients of erythrocyte ALT increased less during vitamin B-6 deficiency than any other activity coefficients. At day 0, the deficient groups had activity coefficients of 1.27 (males) and 1.16 (females) compared to 1.06 and 1.05 for the respective non-deficient groups (footnote, Table 17). Despite these small absolute differences, ANOVA had revealed this parameter to be sensitive to vitamin intake on all sample days except day 28 in the females (Table 15). Significant differences were detected between diet groups by Newman-Keuls only on days 14 and 28 in the males (marginal vitamin level); the frozen group had the lowest, and the semi-purified, the highest activity coefficients. However, the importance of these differences is doubtful because all means were at or below the level of the non-deficient controls.

DISCUSSION

Comments Concerning Sensitivity of Experimental Parameters

The sensitivity of our test system was limited by the stipulation in the protocol that the test meats be fed at 35% of dry weight of the diet. At this level, the meats contributed to the diets 1.2 to 2.4 mg vitamin B-6 per kg diet. Therefore, the marginal repletion level in the study could not be less than 2.4 mg/kg of diet. In the pilot study, we had found 2.0 but not 4.0 mg/kg to be suboptimal relative to 12.0 mg/kg. The low vitamin level selected in the major study (2.5 mg/kg) supported growth responses which were no different than responses to 12.0 mg/kg of diet. However, the low vitamin level was truly marginal as revealed by the distinctly slower recovery rates of all enzyme parameters.

Vitamin B-6-dependent processes involved in growth (weight gain) took priority over the blood aminotransferases during repletion. The low vitamin diets contained enough pyridoxine to support maximal growth response, but recovery rates of the aminotransferases were clearly slower when compared to the high vitamin groups. Thus the blood enzymes were more sensitive indicators of vitamin B-6 intake than was growth.

Aminotransferases as Indicator of Vitamin B-6 Status

In this study, ALT measurements were more sensitive to vitamin B-6 intake than AST parameters. This observation has been documented for the plasma enzyme for both rats (2) and humans (19). In addition, rat ALT appears to be more responsive than AST to other "physiological stresses" (20,21). Neither AST nor ALT in plasma is considered useful

in assessing vitamin B-6 nutriture in the human because of the wide range of activity in normal individuals (3,19). In experimental animals, however, plasma ALT appears to be an excellent parameter. Furthermore, in our study, both erythrocyte aminotransferases were highly sensitive to vitamin B-6 intake.

Of the enzymatic paramaters employed, plasma AST was the least sensitive to vitamin B-6 intake. After the first week of repletion, there was no consistent difference between the high and low vitamin intake groups in either plasma AST or its activation coefficient. In contrast, both erythrocyte AST and its activation coefficient did distinguish between the vitamin levels on all sample days in both sexes (with one exception). However, from the second week on, all repletion groups had returned to, or surpassed, the levels of the groups which had never been deficient (Figures 9 and 10). Thus, the significant effect of vitamin intake from day 14 on was largely due to the fact that the high vitamin groups had exceeded the enzymatic activity levels of the controls (which had been maintained on an intake of 12 mg/kg all along). The cause and significance of this overshoot are not known.

Plasma and erythrocyte ALT were the most sensitive parameters in our study. In the deficient animals, ALT was more markedly depressed than AST in both plasma and erythrocytes. During repletion, not only was the effect of vitamin intake obvious on all sample days in both sexes, but the magnitude of the differences was greater than was found in the AST measurements.

The overshoot phenomenon occurred with erythrocyte AST and to an even greater degree with plasma ALT. This was particularly striking among all high vitamin groups, both male and female, for the first two weeks (Figures 17 and 18). By day 28, these group means were nearly back to the levels of non-deficient controls. In contrast to the high vitamin groups, all marginal repletion groups showed a large increase in plasma ALT only during the first week and changed little thereafter. By the fourth week, the marginal groups still had lower ALT activity than the high vitamin groups and the non-deficient controls.

Plasma ALT activity coefficients of male high vitamin groups also tended to be less than the non-deficient levels, especially during the first 2 weeks. Thus the overshoot phenomenon was observed, not only in terms of absolute enzymatic activity, but also in the relative saturation of apoenzyme with cofactor. Activity coefficients did not overshoot in the females. This may be related to the fact that in the non-deficient animals, the apoenzyme appears to be more saturated with cofactor in the females than in males (Table 14).

Erythrocyte ALT not only did not overshoot non-deficient levels, but it was the only activity not to be restored within the 4-week repletion period. On day 28, the high vitamin groups were nearly back

To the non-deficient levels, but the marginal vitamin groups had recovered only approximately half way. Presumably, the slower rise in activity was due in part to the time required for the deficient cells to be replaced during red cell turnover with erythrocytes containing higher ALT levels.

Erythrocyte ALT activity coefficient was not impressive as an indicator of vitamin B-6 intake. Although analysis of variance revealed significant vitamin intake effects on 5 out of 6 observation days, the magnitude of the differences was small compared to other parameters.

Vitamin B-6 Assay

The vitamin assay presented the most challenging problems of this study. The organism of choice was the vitamin B-6-dependent yeast, Saccharomyces uvarum, which responds equally to pyridoxine, pyridoxal, and pyridoxamine under ideal conditions (8). Under less than ideal conditions, however, the organisms will not necessarily respond equally to all 3 forms of vitamins B-6 or their mixtures. The assay medium is formulated to contain adequate amounts of all nutrients so that growth response to the test substance is due solely to the vitamin B-6 content. If the medium is not complete in all other respects, two problems may occur: a) false high values for test samples and b) "upward drift," i.e., nonlinearity of the response to test samples with respect to the standard curve. We experienced both of the above problems.

A commercial medium (Pyridoxine Y medium, Difco Laboratories, Detroit, MI) was used for the first assays including the estimates of the vitamin contents of the test meats (Table 3). Upward drift was a recurring problem and was as much as 200% with a 4-fold difference in dilution. Because of the drift problem and day-to-day variability, our confidence in the test meat vitamin values leaves much to be desired.

We next prepared the medium recommended by Sauberlich (8) and found that it promoted greater growth than did the Difco medium. Upward drift was reduced but was not eliminated. Drift was further decreased by additional supplementation of the medium with lysine, leucine, and methionine. All remaining assays (diets) were done with this supplemented medium.

According to the microbiological assay, the marginal diet based on frozen chicken contained approximately 30% more vitamin B-6 than other meat-containing diets (Table 1). This occurred despite the fact that all diets had been carefully formulated to contain identical amounts of vitamin B-6. A possible explanation for the discrepancy is as follows: most (94%) of the vitamin B-6 in the frozen chicken diet originated in the chicken. Most of this vitamin B-6 was probably protein-bound (e.g., pyridoxal phosphate). The form of the vitamin in the other three diets was 22 to 45% crystalline pyridoxine. Free pyridoxine would be expected to be more labile than the protein-bound forms. Some degradation likely occurred during the routine mixing of the moist test meats with dry

pre-mixes (exposure to moisture, light, and air at room temperature). Greater destruction of pyridoxine in the thermal, gamma, and electron diets could have accounted for the decreased vitamin B-6 content in these diets. Assayable vitamin B-6 was similar in all high vitamin chicken diets. This is not surprising because all contained relative excesses of added pyridoxine.

Effects of Diet on Enzymatic Parameters

Brin and co-workers have reported that "rats fed X-irradiated pork were subject to marginal deficiency of pyridoxine" (22). Theirs was not a depletion/repletion study, but instead a simple feeding study in which rats were fed (frozen) control pork with and without pyridoxine supplementation and X-irradiated pork (35% dry weight). After 12 weeks, serum ALT was highest in the group fed supplemented pork, significantly lower in the unsupplemented control group, and lower yet in the unsupplemented irradiated group. (There was no irradiated + vitamin B-6 group.) The results were completely consistent with the assayable vitamin B-6 contents of the meats. Thus, the "marginal deficiency of pyridoxine" would be detected only when the diets are formulated to contain no additional source of the vitamin.

In the present study, there was no one diet group which was dramatically and consistently different from all others in terms of enzymatic responses to repletion. There were however, trends; e.g., among the marginal vitamin groups in general, the group fed frozen chicken responded the best, and those fed the semi-purified diet the poorest. The lower responses during repletion with the semi-purified diet can be explained by the lower food consumption. At equivalent intakes, this diet should have promoted responses equivalent to the meat-containing diets. Without a paired feeding study, however, it is difficult to compare responses on the semi-purified diet to the chicken diets. The chicken-based diets were consumed at approximately equal amounts and growth rates were essentially identical for all of them.

The frozen control group usually had the highest enzymatic activities and lowest activity coefficients among the marginal groups. This is consistent with the slightly higher vitamin B-6 content of the frozen (marginal) diet, which was discussed above. The marginal vitamin groups fed thermally processed and electron irradiated chicken fared almost as well as the control group (frozen chicken) and were statistically different in only a few cases. The group fed gamma irradiated chicken tended to respond the slowest of the meat groups.

The enzyme data could support a conclusion that the diets containing gamma irradiated chicken promote slower repletion of vitamin B-6-deficient rats than diets containing frozen chicken. Differences between these groups, especially in plasma and erythrocyte ALT activities, were statistically significant a sufficient number of times to allow such a generalization if other factors were not considered.

However, we hesitate to conclude that gamma irradiated chicken contains an antivitamin B-6 factor(s), or if such a factor does exist, that it is a biological hazard. Our reasons for skepticism are:

- The experimental design required that the marginal diets contain identical suboptimal levels of vitamin B-6. To accomplish this, it was necessary to assay the test meats in advance and to supplement each diet accordingly. Unfortunately, the best available assay for the total composite of vitamin B-6 in natural materials, such as meat, utilizes a living organism. Any compound which is antagonistic toward vitamin B-6 in rats might just as well behave similarly in yeast. If this is the case, the study is self-defeating. We cannot be sure whether the lower assayable vitamin B-6 in irradiated chicken was due to destruction of the vitamin, production of an antagonist, or both. It is also hypothetically possible (but not easily tested) that gamma irradiation converts the vitamin to a form less available in the rat but still utilized by S. uvarum. We feel these considerations represent serious flaws in the original protocol.

- The magnitude of the enzyme effects and the conditions for detecting them suggest that an antivitamin B-6 factor (if present) is not biologically important. The absolute differences between gamma irradiated and control groups were small when compared to the magnitude of the changes in each group between days 0 and 7. Among the high vitamin groups, few diet effects were observed and they did not fit a pattern. Thus, the delayed responses to repletion in the animals fed gamma irradiated chicken were detectable only under conditions of marginal vitamin B-6 intake and were easily overcome by additional dietary pyridoxine.

If gamma irradiated chicken does contain an antivitamin B-6 compound, it should not be of great consequence under normal circumstances because it is a minor component and other constituents of the diet would contribute enough vitamin B-6 to compensate for the decreased vitamin in the chicken.

CONCLUSIONS

- Vitamin B-6 deficient rats were repleted with semi-purified diets or diets containing chicken (frozen, thermally processed, gamma, or electron irradiated). No difference was found in growth response (weight gain) among the chicken-fed groups. The groups fed semi-purified diets responded slower, presumably because of lower food consumption.

• ALT (both in plasma and erythrocytes) was a better indicator of vitamin B-6 status than AST under the conditions of the present study. The magnitude of the differences between deficient and control values was greater for ALT than AST. ALT parameters were not only slower to recover, but their recovery rates were more dependent upon vitamin B-6 intake than were the corresponding AST parameters.

• The enzyme responses of rats fed frozen, thermally processed, and electron irradiated chicken were similar. Responses of some of the enzymatic paramaters were slightly delayed in groups fed gamma irradiated chicken at the marginal vitamin B-6 level. No differences were observed at the high vitamin level. Similar results were obtained with both male and female rats.

• No evidence was found for antivitamin properties in electron irradiated chicken. The amount of antivitamin B-6 activity in gamma irradiated chicken (if indeed present) is minimal and probably not important enough to offset advantages of food preservation by irradiation.

RECOMMENDATIONS

None

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LIST OF FIGURES

APPENDIX A

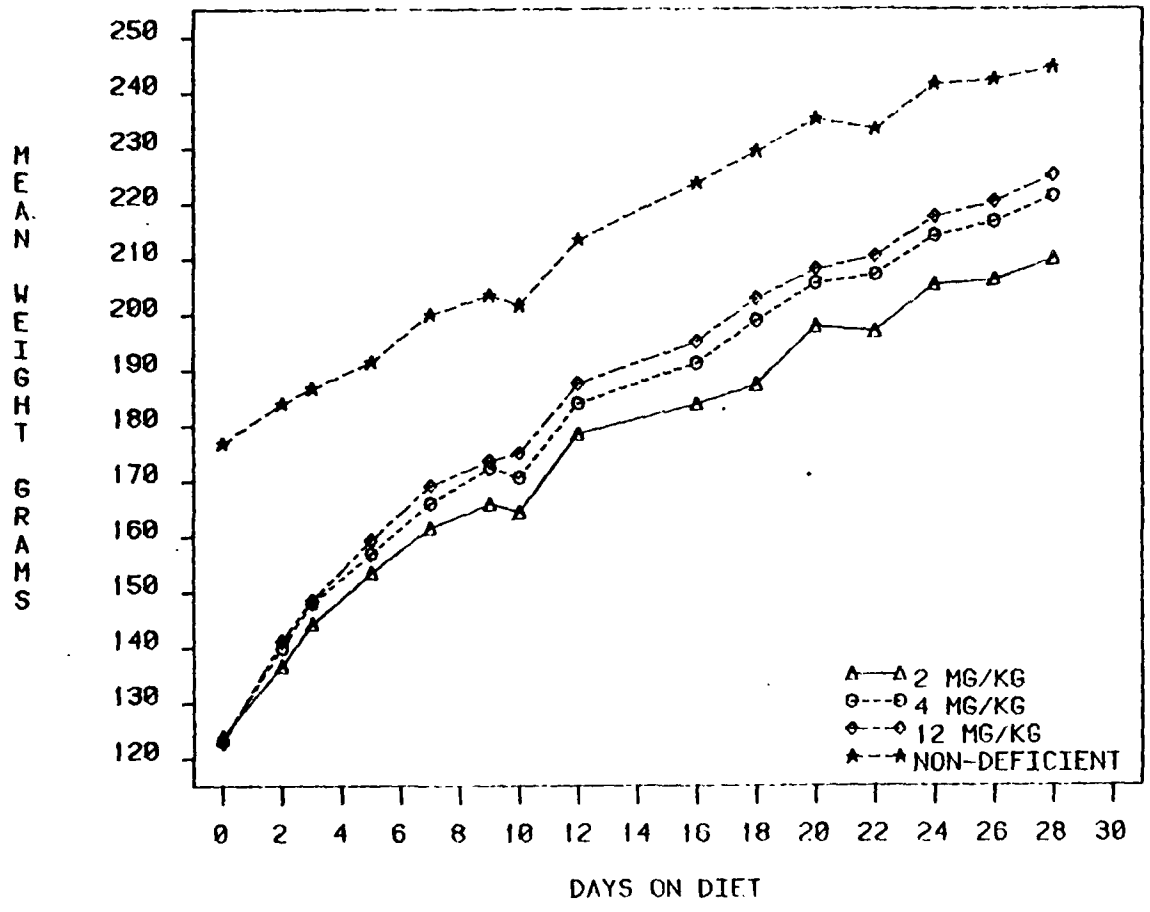


Figure 1. Growth responses of vitamin B-6 deficient rats (females) repleted at 3 levels of dietary pyridoxine (pilot study).

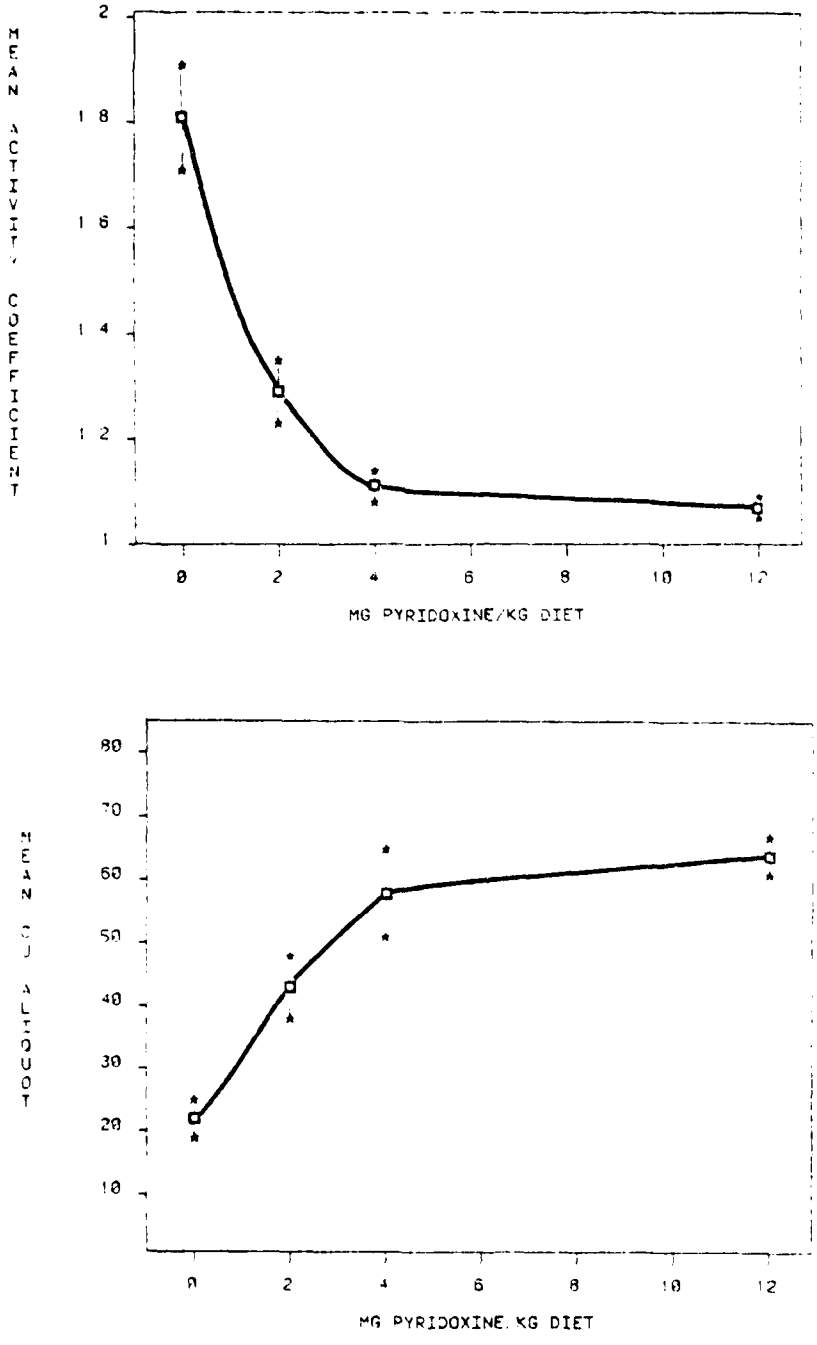


Figure 2. Erythrocyte aspartate aminotransferase responses of vitamin B-6-deficient rats (females) repleted one week at 3 levels of dietary pyridoxine. Upper graph, activity coefficient, lower graph, enzymatic activity in (arbitrary) chart units. (Pilot study). Vertical bars represent mean ± S.D.

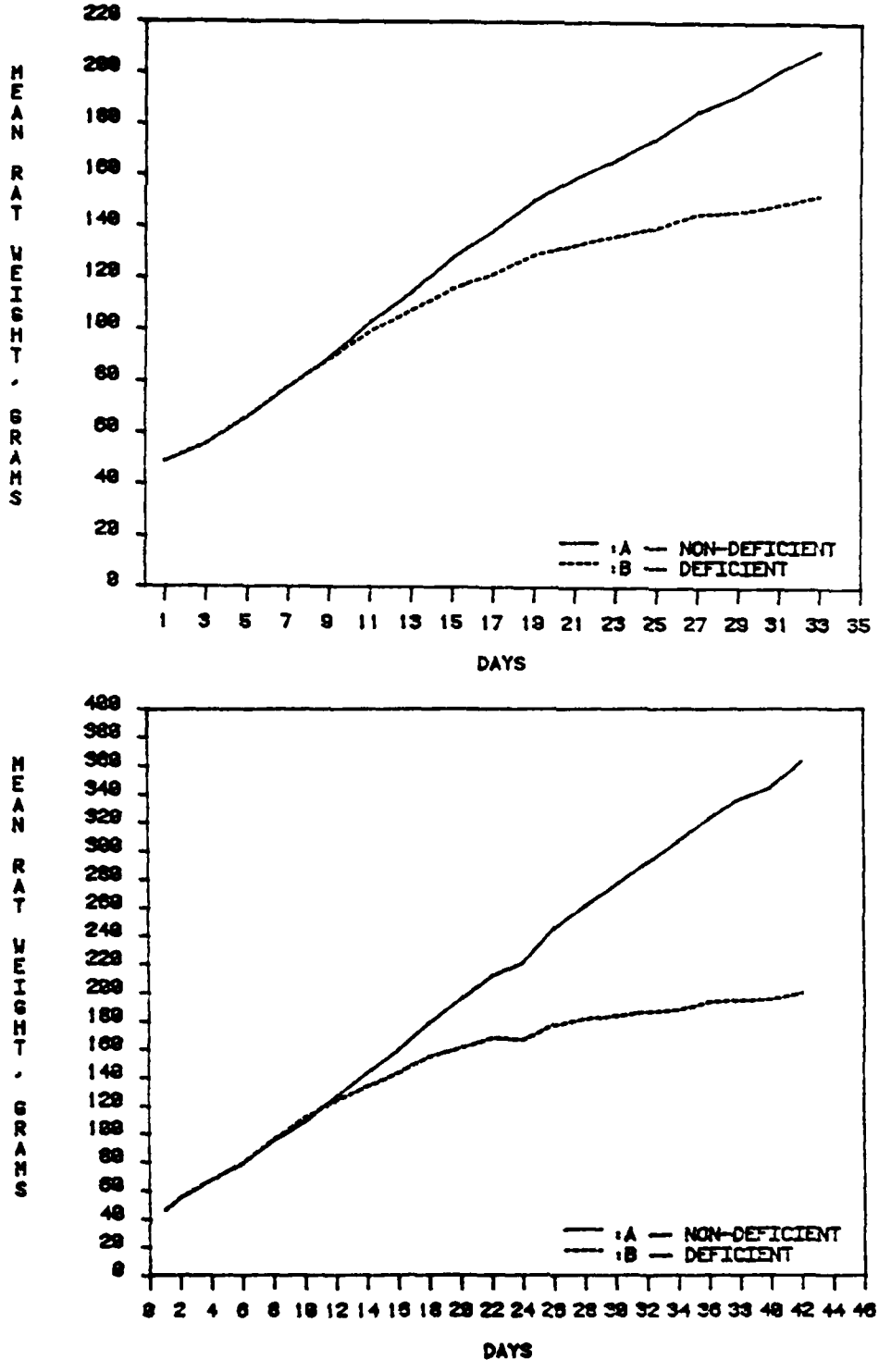


Figure 3. Growth curves, phases 1 and 2. Females (upper), males (lower).

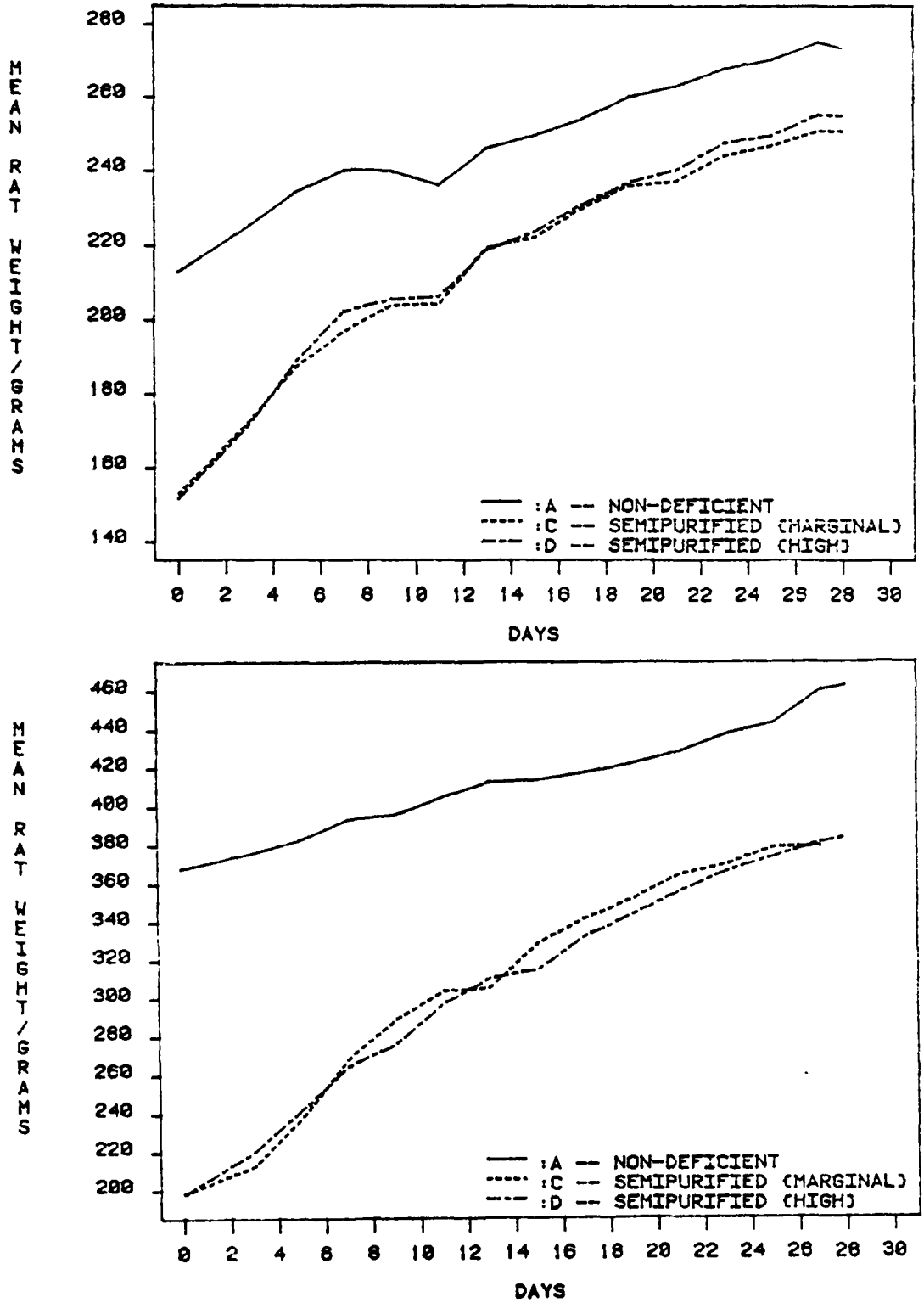


Figure 4. Growth curves, phase 3, Groups C and D. Females (upper), males (lower).

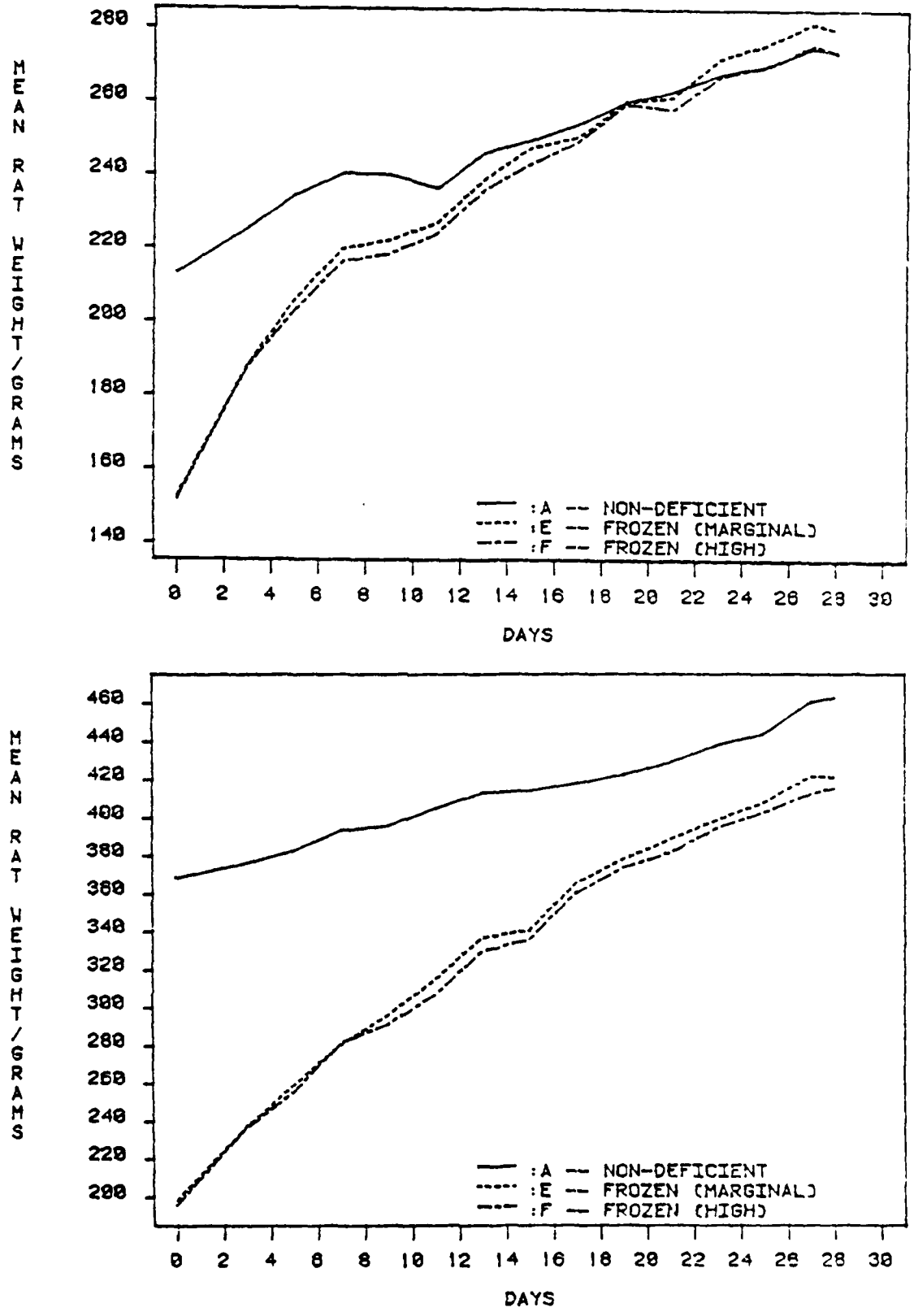


Figure 5. Growth curves, phase 3, Groups E and F. Females (upper), males (lower).

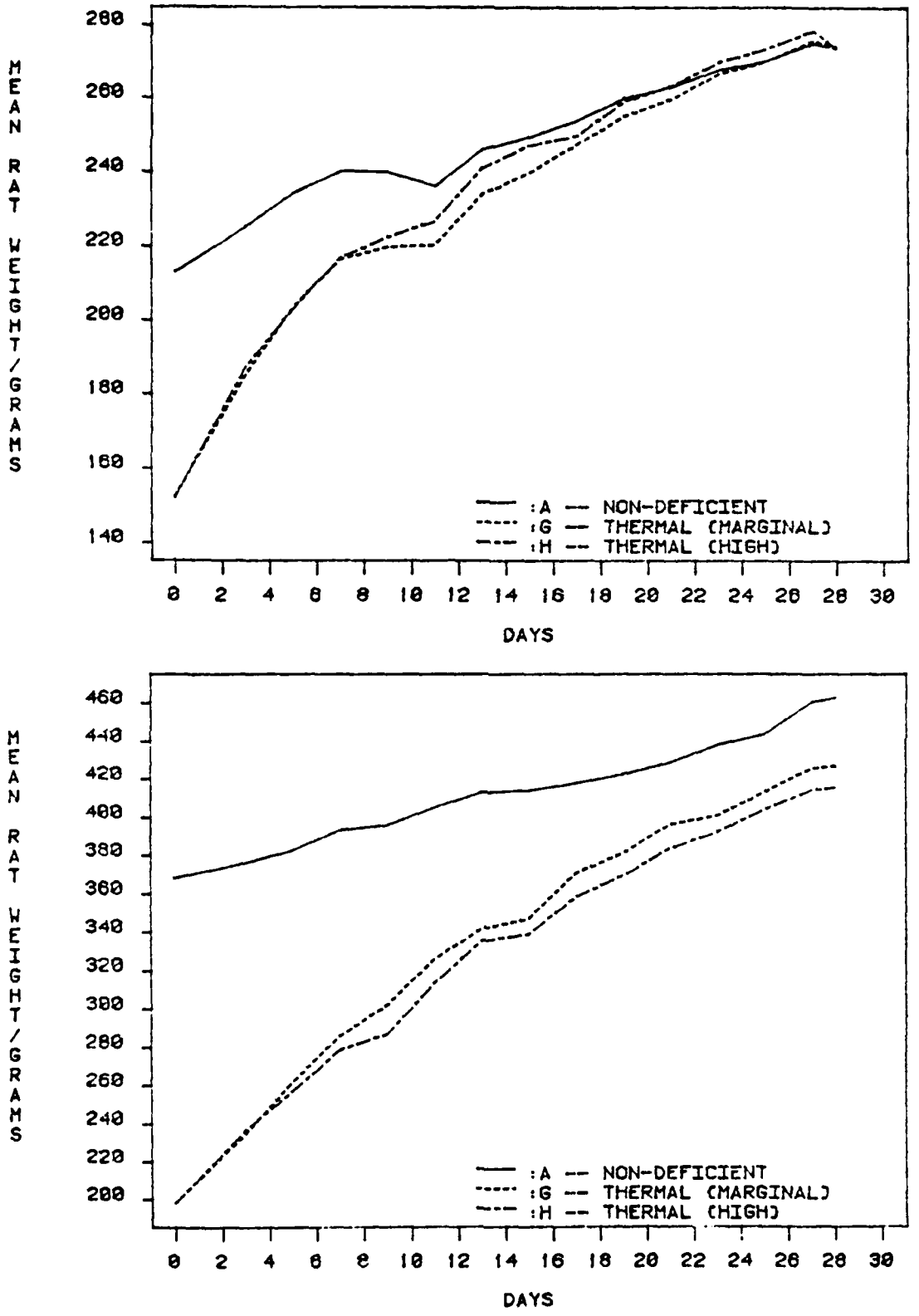


Figure 6. Growth curves, phase 3, Groups G and H. Females (upper), males (lower).

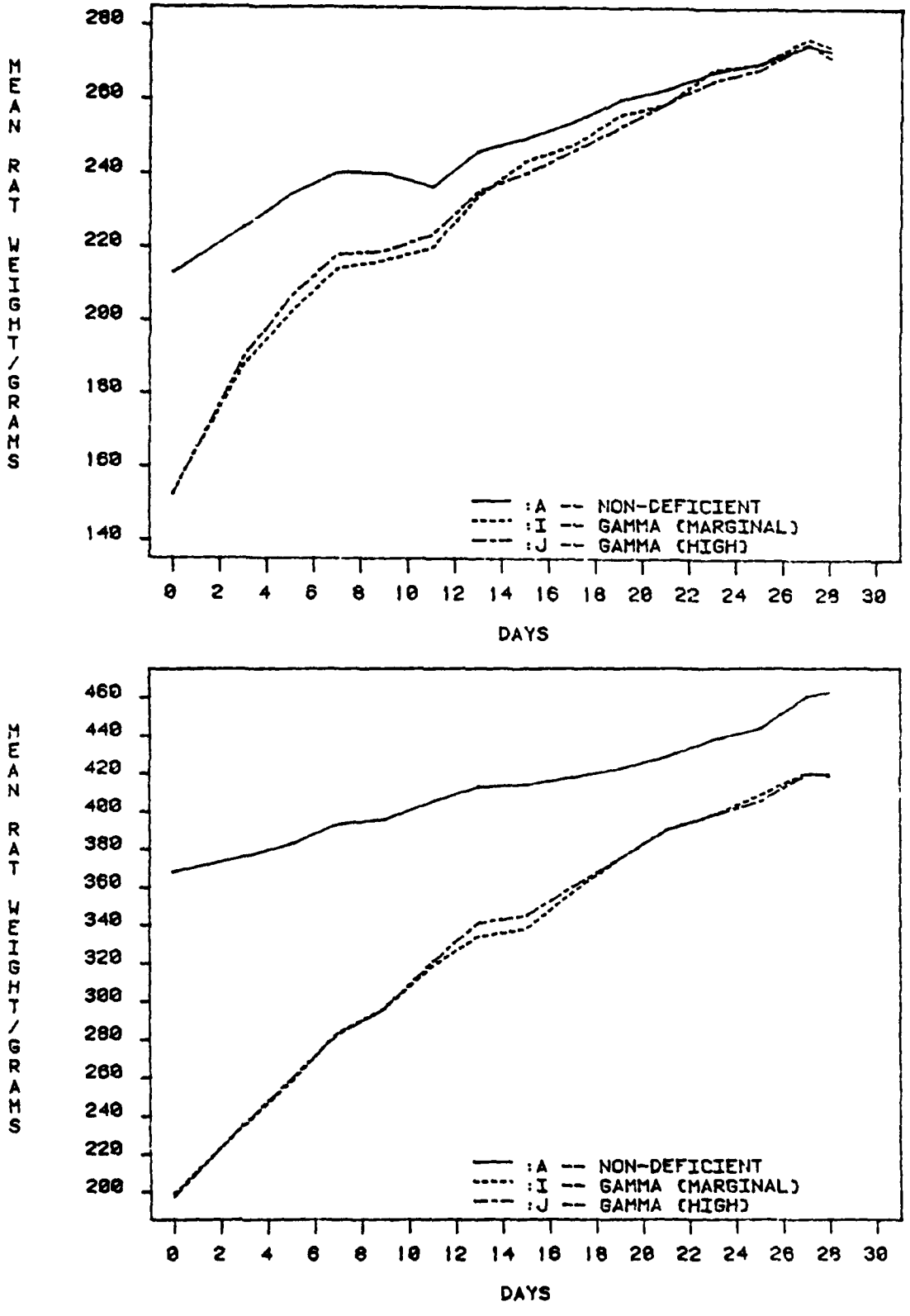


Figure 7. Growth curves, phase 3, Groups I and J. Females (upper), males (lower).

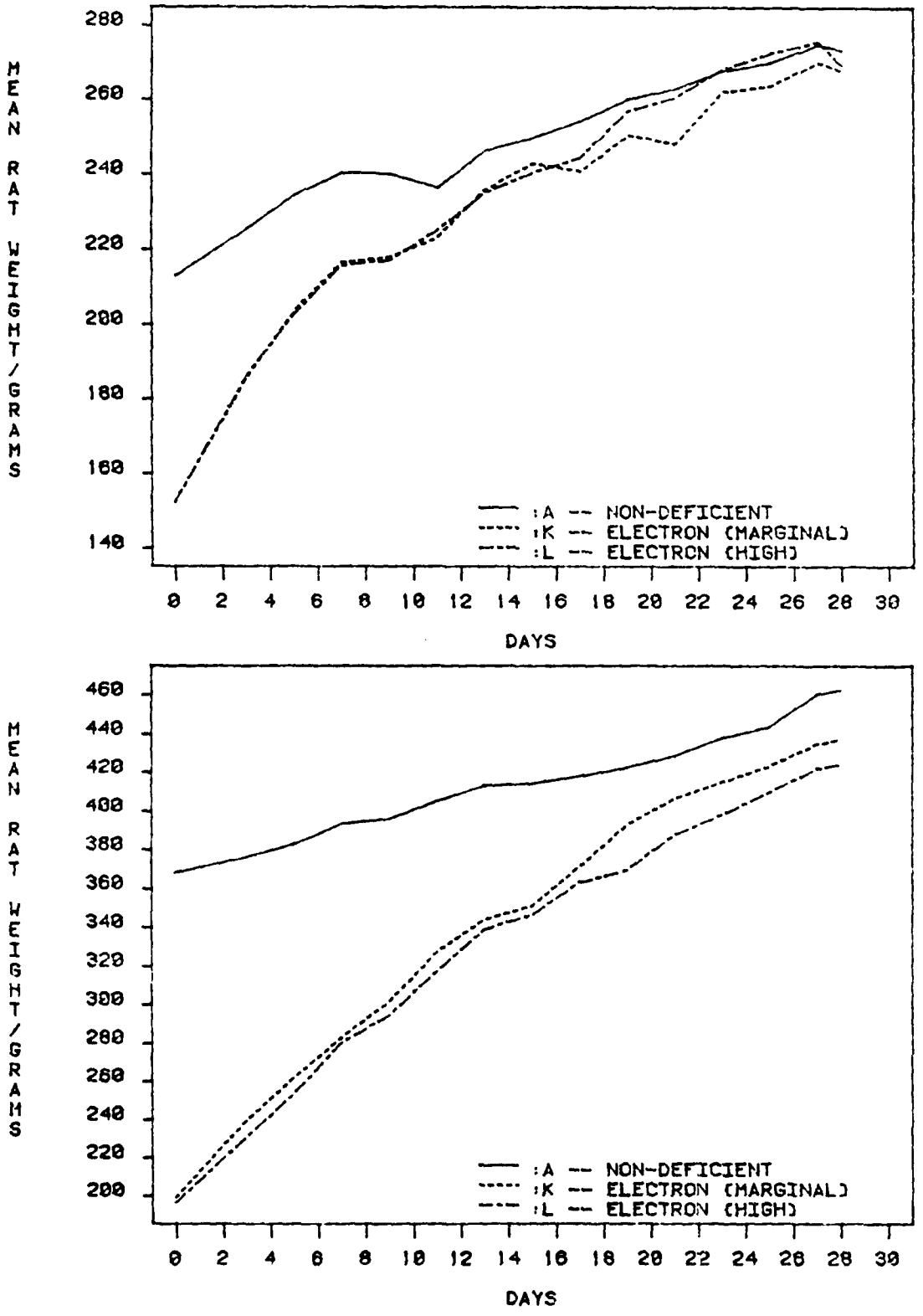


Figure 8. Growth curves, phase 3, Groups K and L. Females (upper), males (lower).

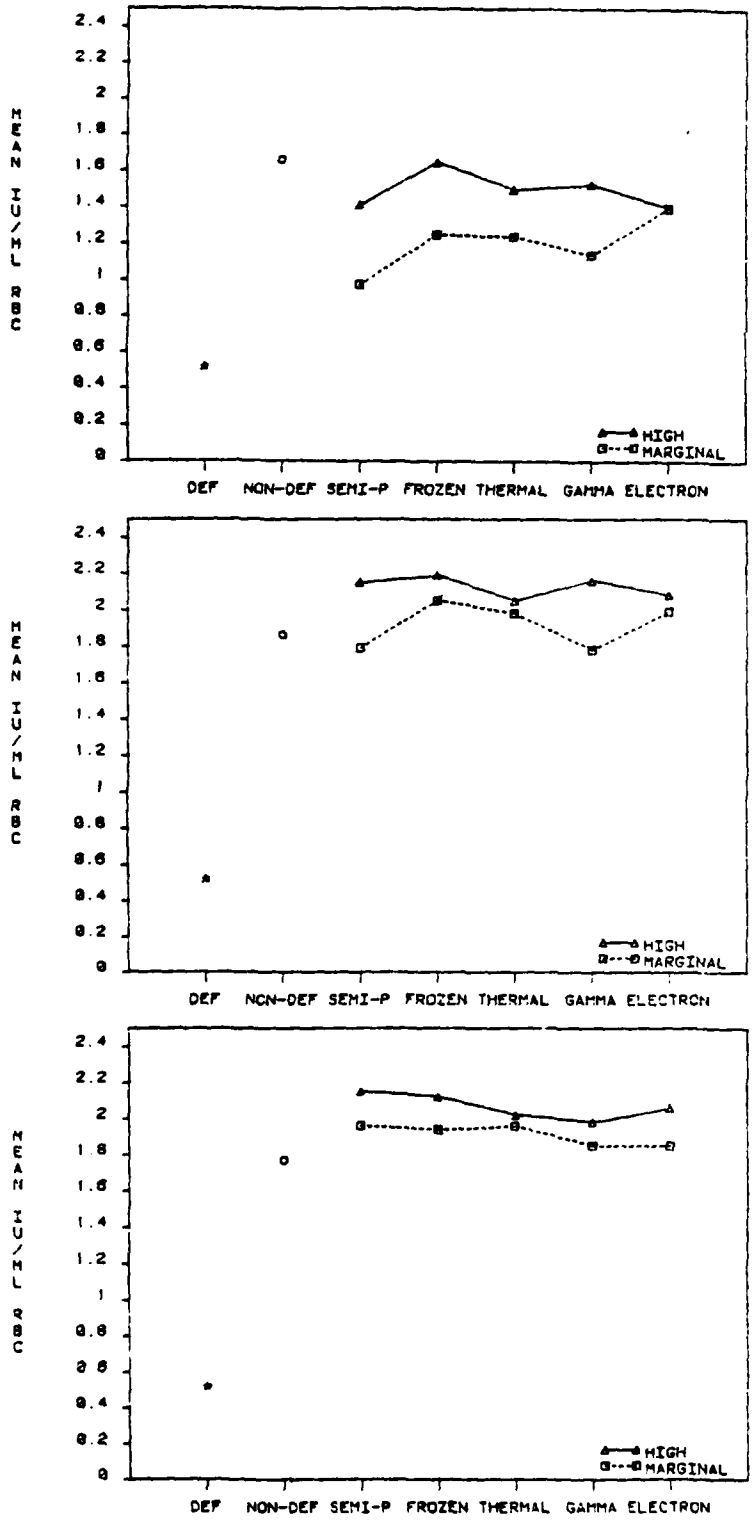
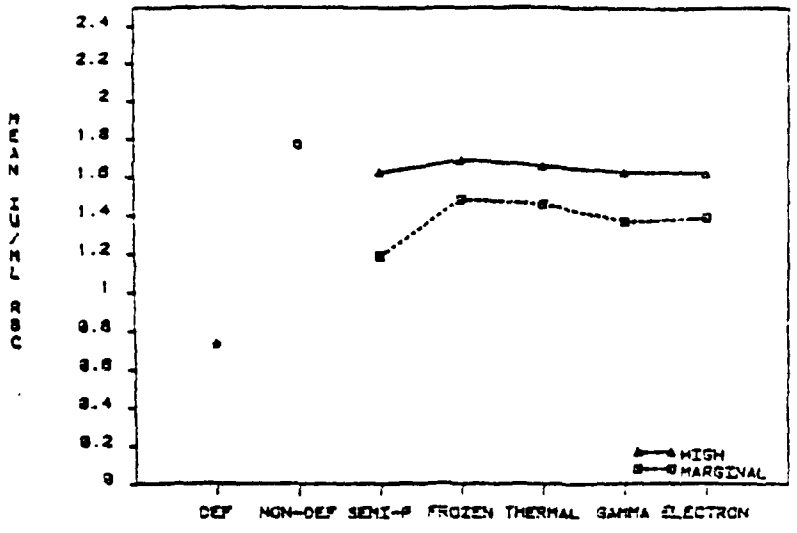
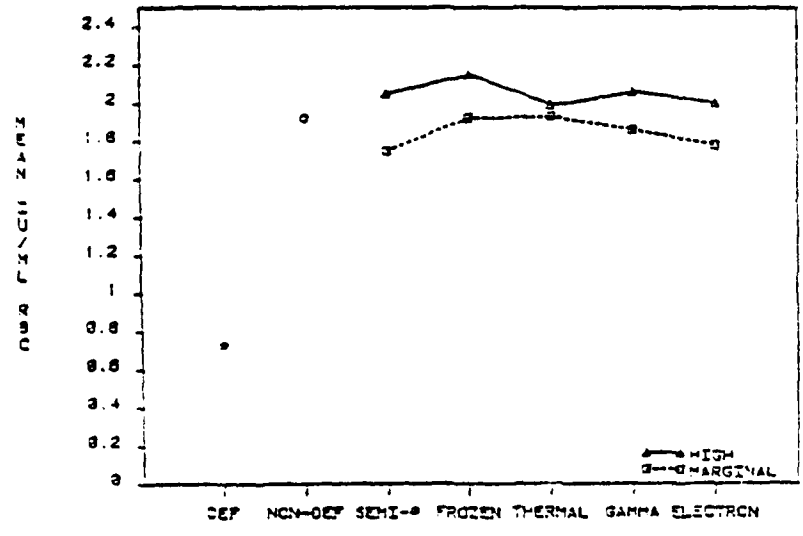


Figure 9. Erythrocyte aspartate aminotransferase activity, males (Group means) Symbol at far left represents deficient group at Day 0.



Day 7



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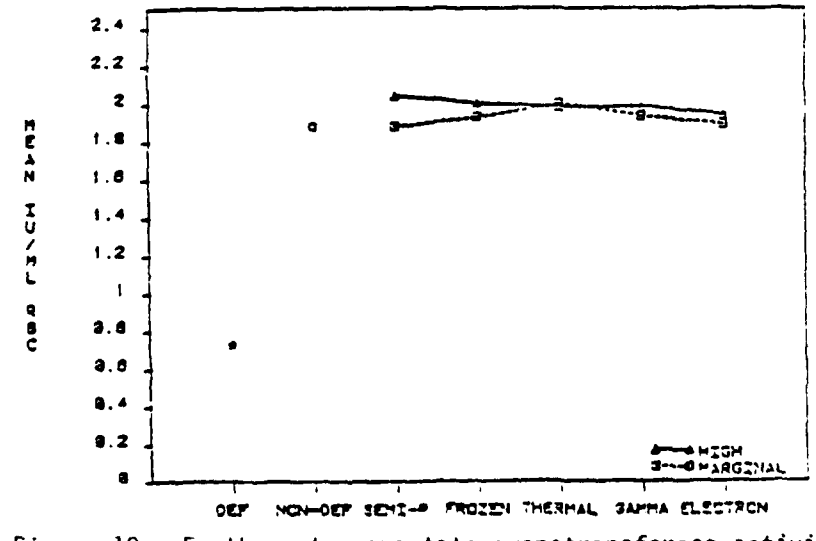
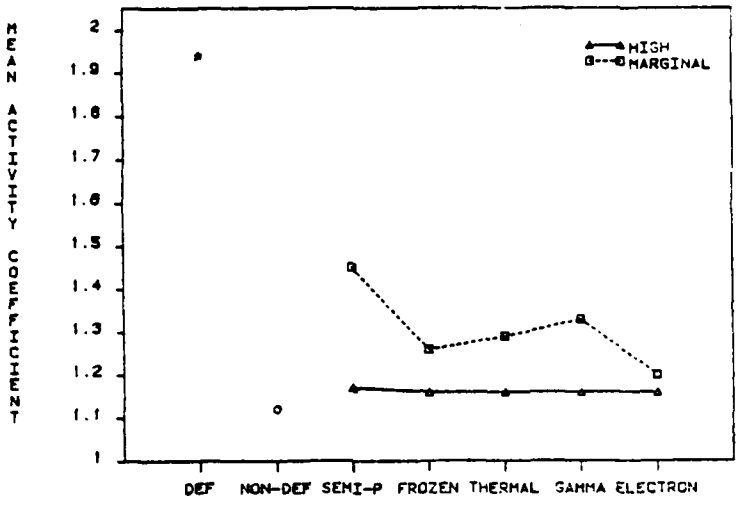
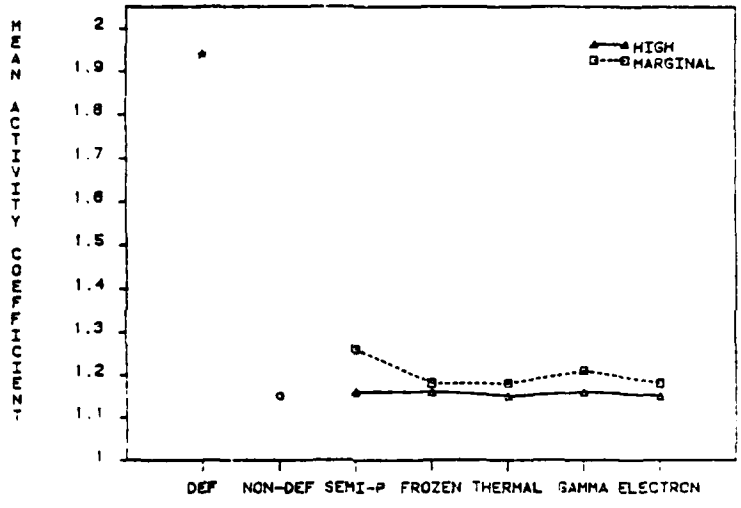


Day 28

Figure 10. Erythrocyte aspartate aminotransferase activity, females. Symbol at far left represents deficient group at Day 0.



Day 7



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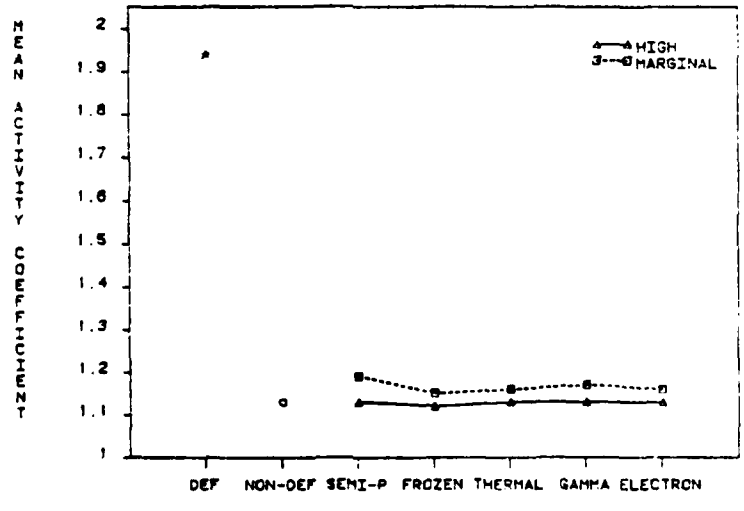
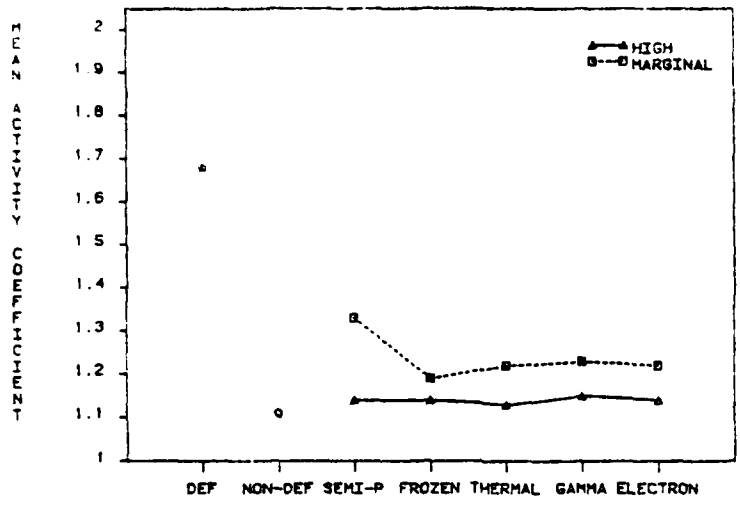


Day 28

Figure 11. Erythrocyte aspartate aminotransferase activity coefficients, males. Symbol at far left represents deficient group at Day 0.



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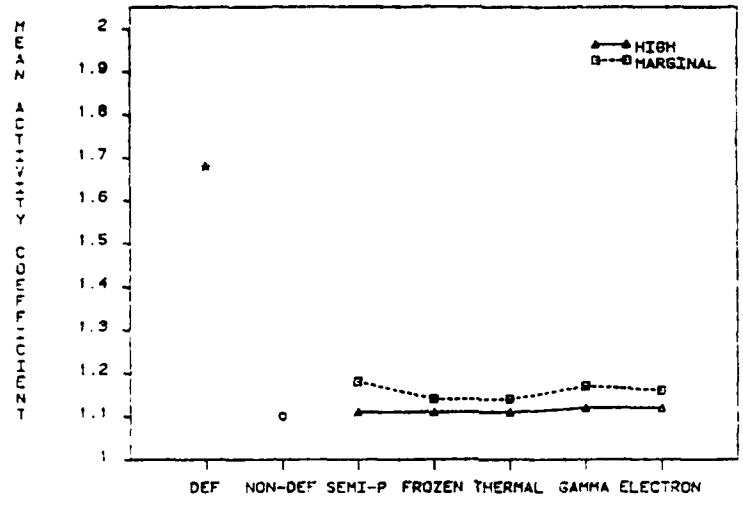
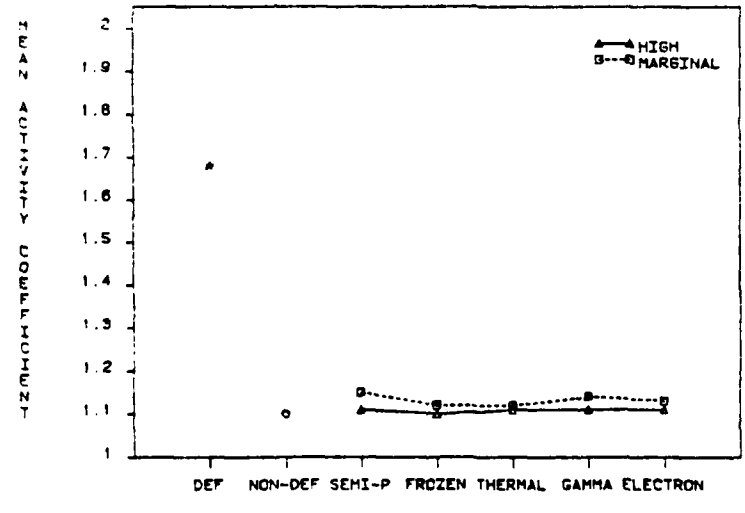


Day 14



Day 28

Figure 12. Erythrocyte aspartate aminotransferase activity coefficients, females. Symbol at far left represents deficient group at Day 0.

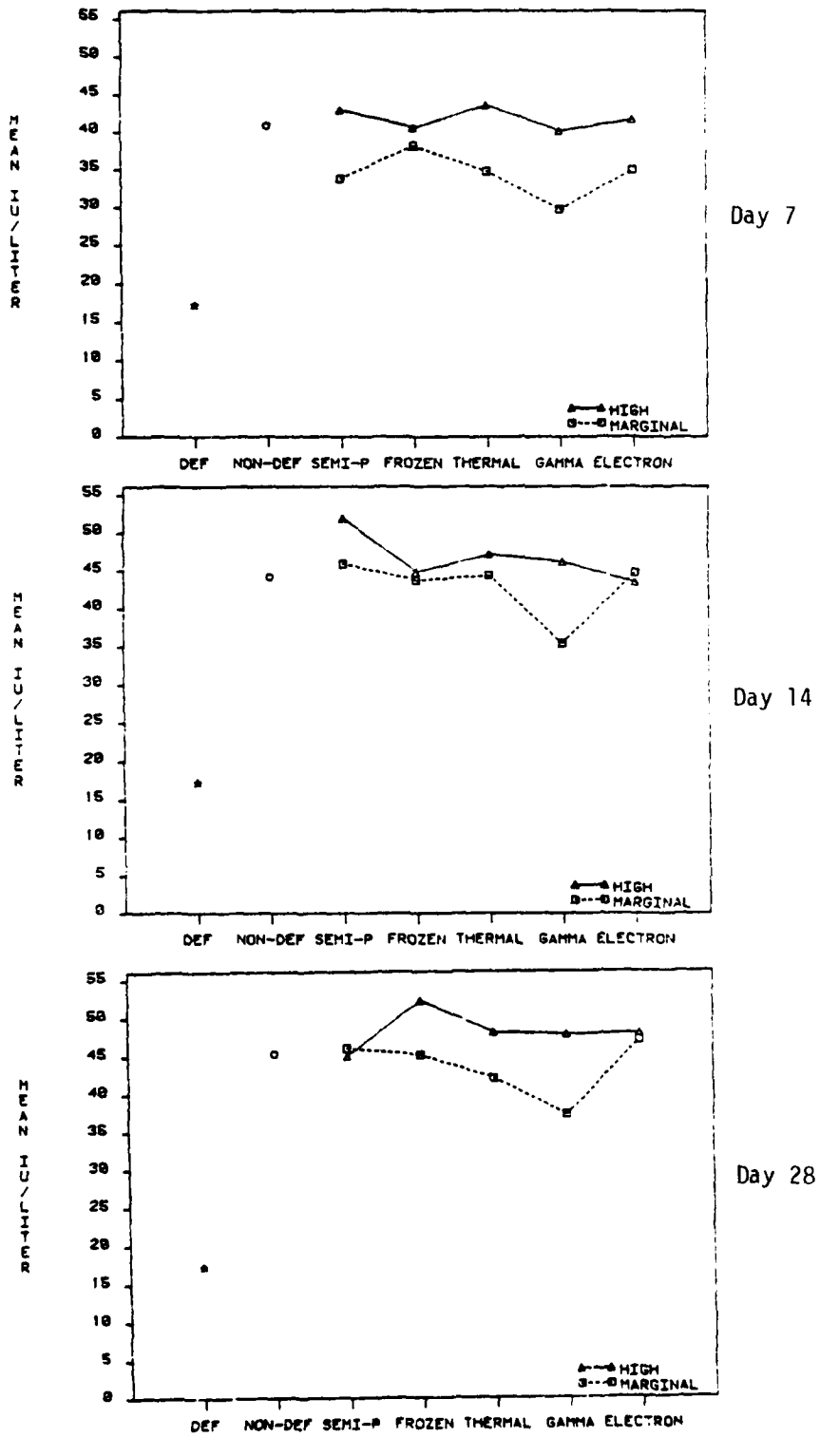


Figure 13. Plasma aspartate aminotransferase activity, males. Symbol at far left represents deficient group at Day 0.

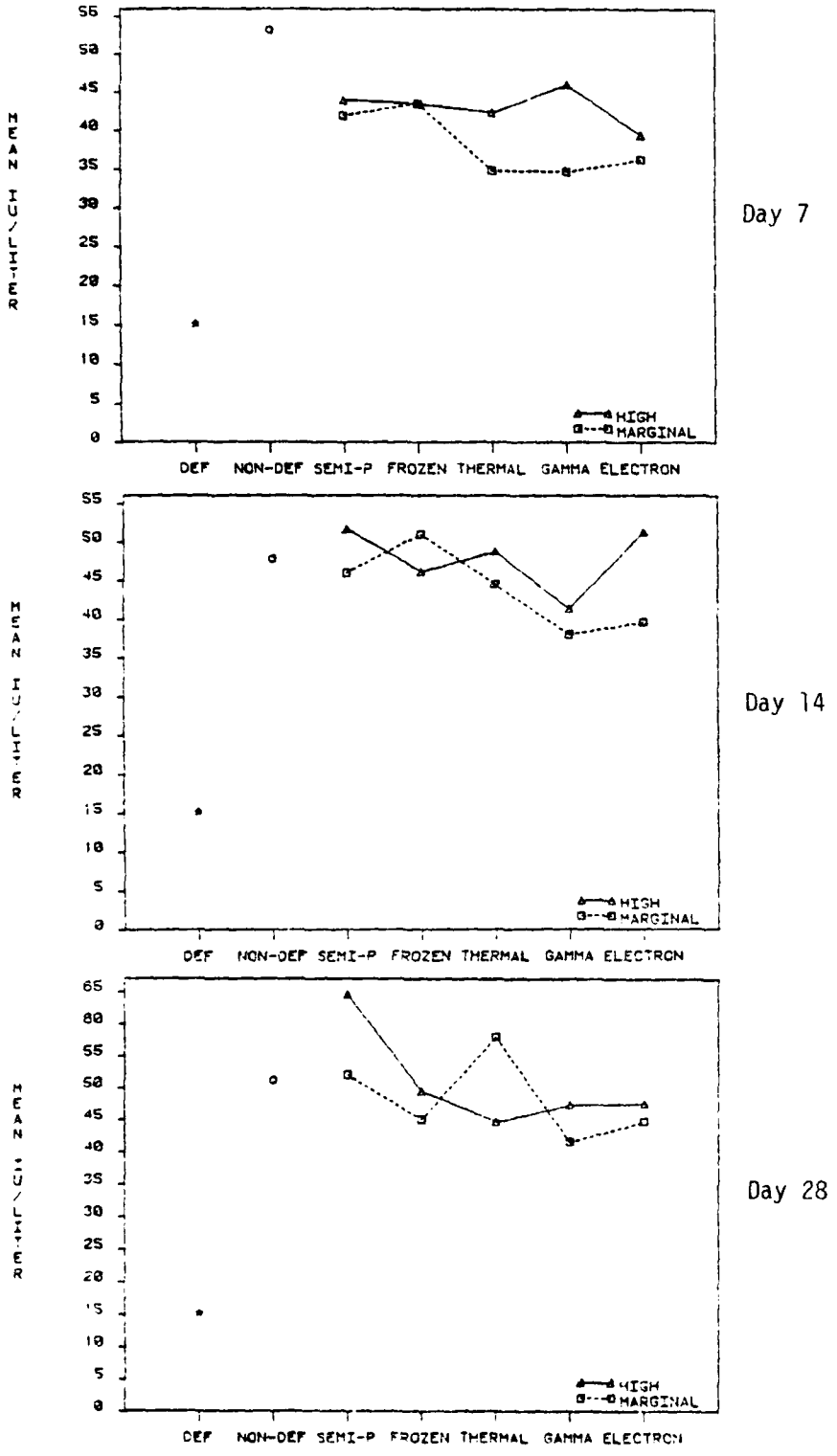
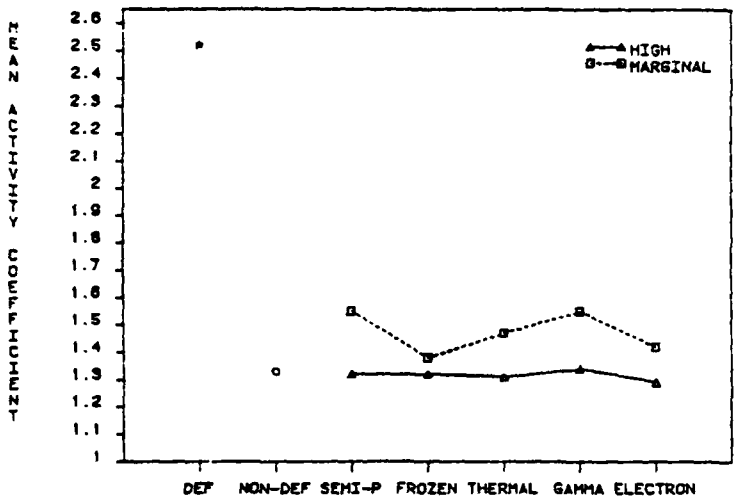


Figure 14. Plasma aspartate aminotransferase activity, females. Symbol at far left represents deficient group at Day 0.



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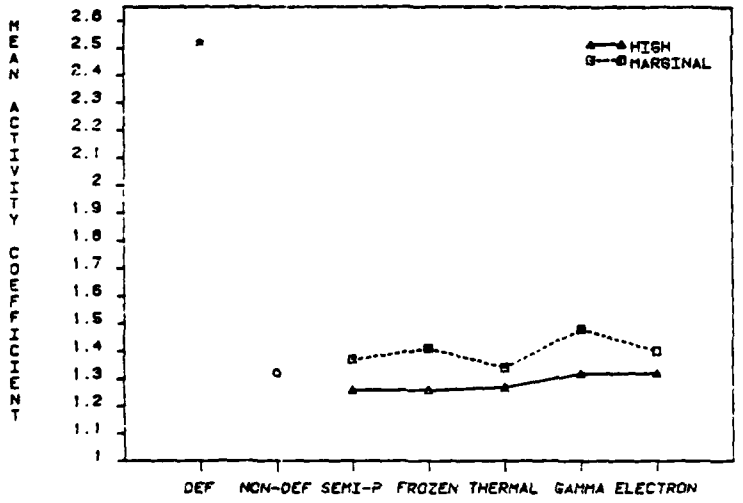
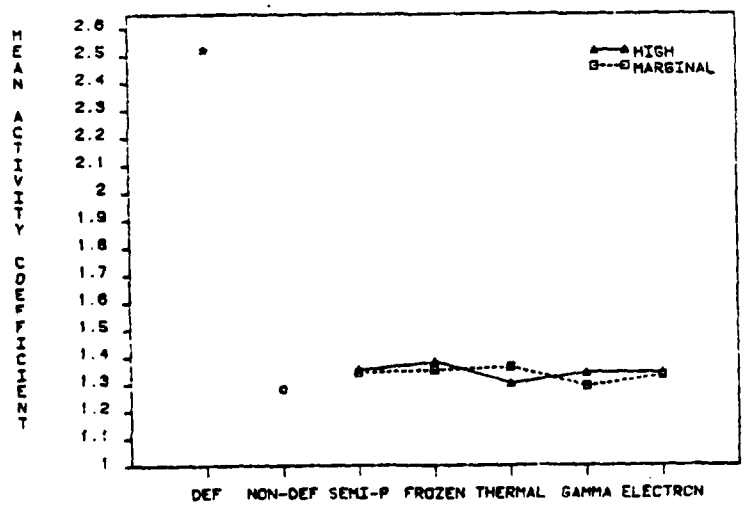


Day 14



Day 28

Figure 15. Plasma aspartate aminotransferase activity coefficients, males. Symbol at far left represents deficient group at Day 0.

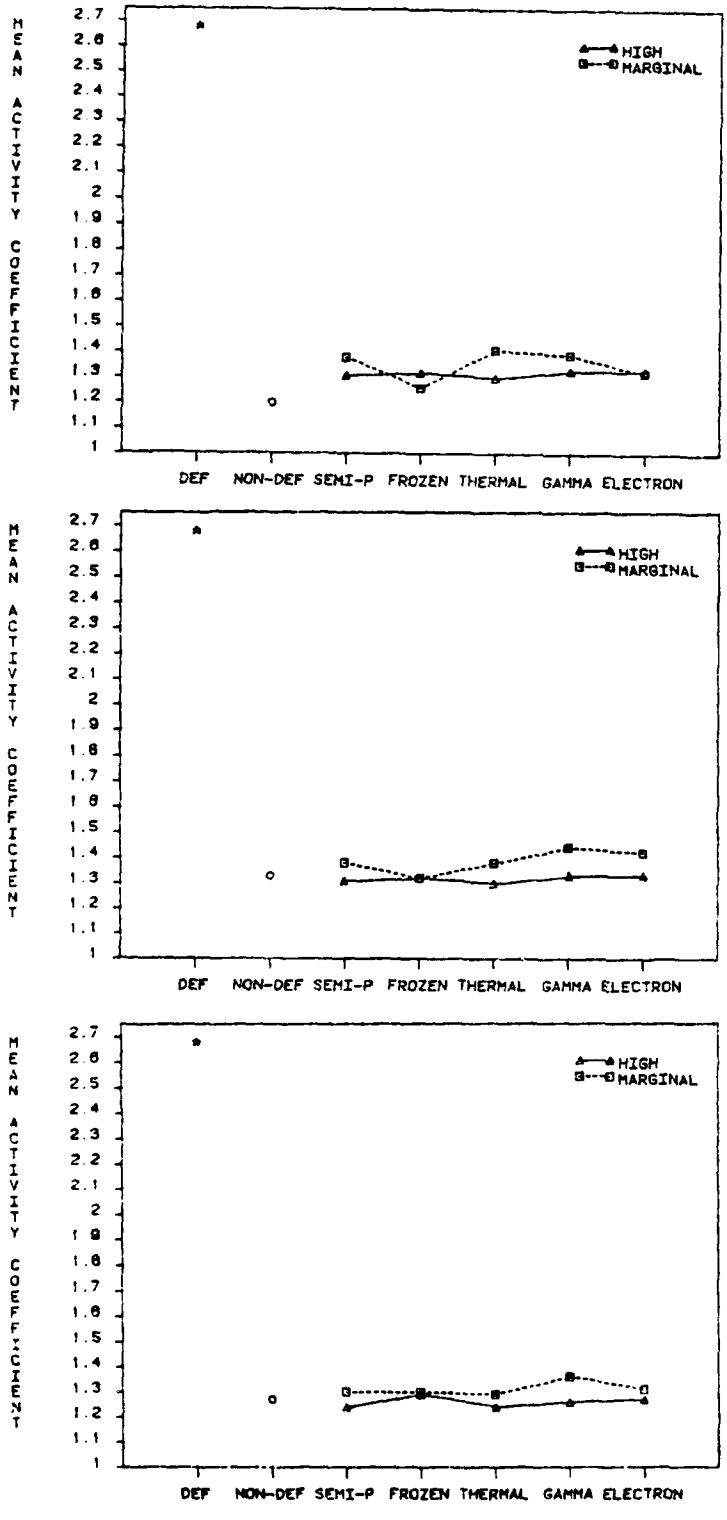


Figure 16. Plasma aspartate aminotransferase activity coefficients, females. Symbol at far left represents deficient group at Day 0.

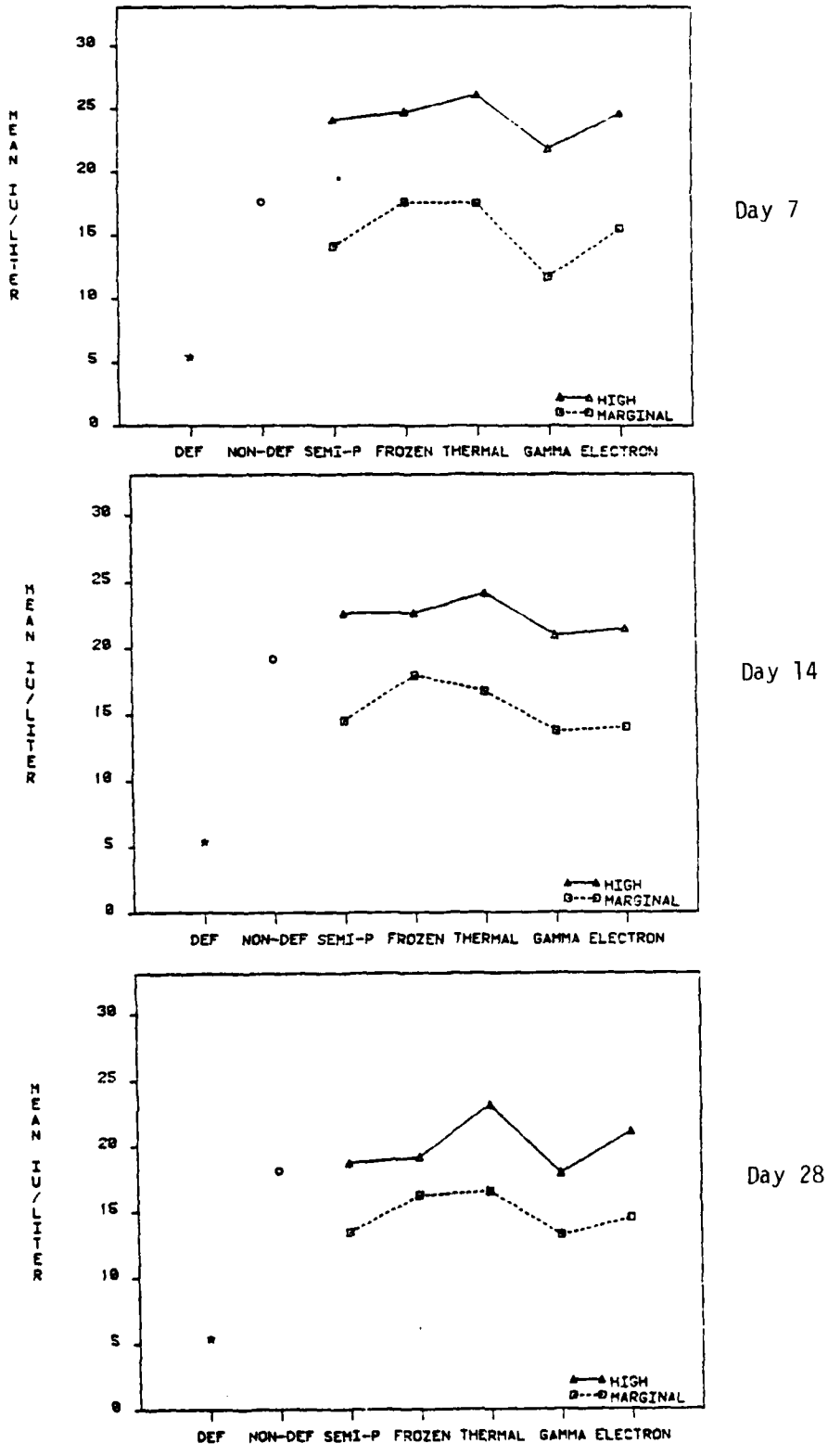
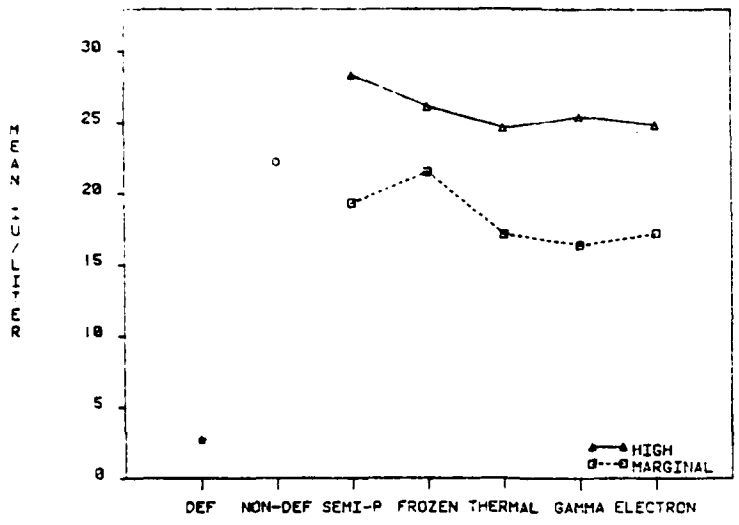


Figure 17. Plasma alanine aminotransferase activity, males. Symbol at far left represents deficient group at Day 0.



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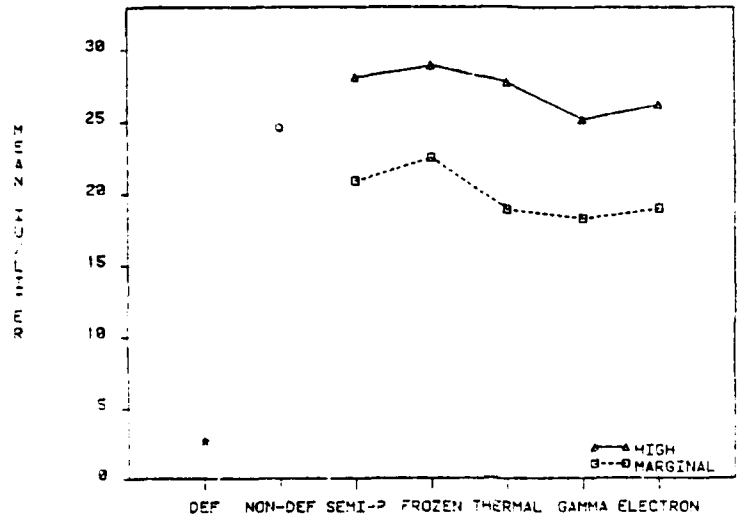

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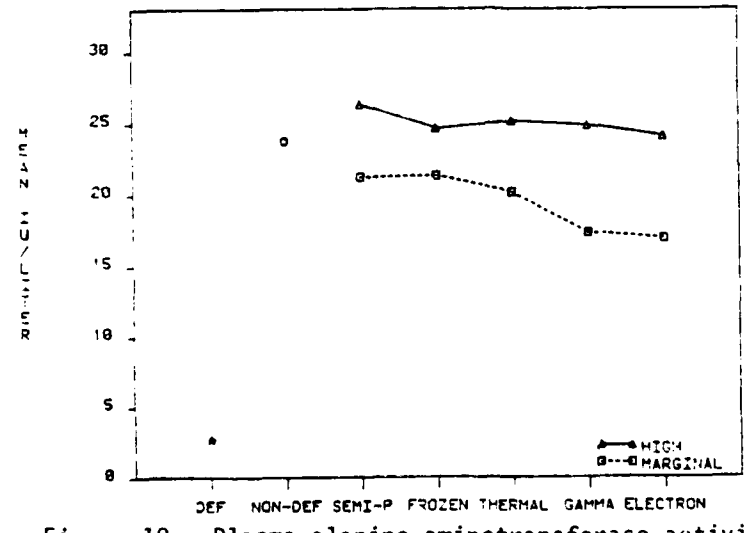
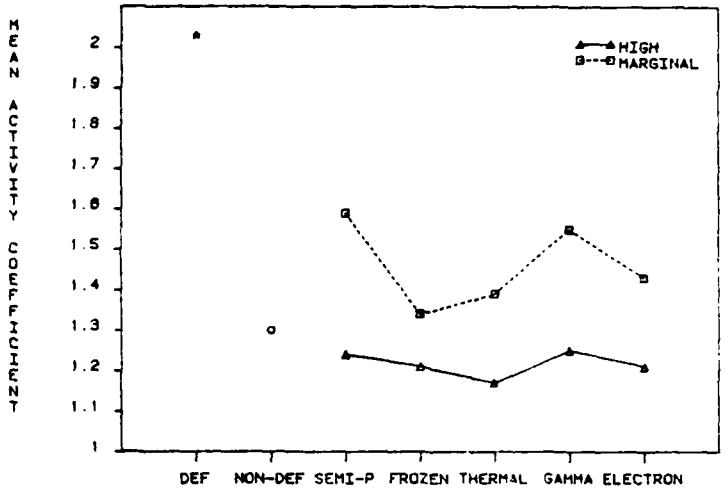


Day 28

Figure 18. Plasma alanine aminotransferase activity, females. Symbol at far left represents deficient group at Day 0.



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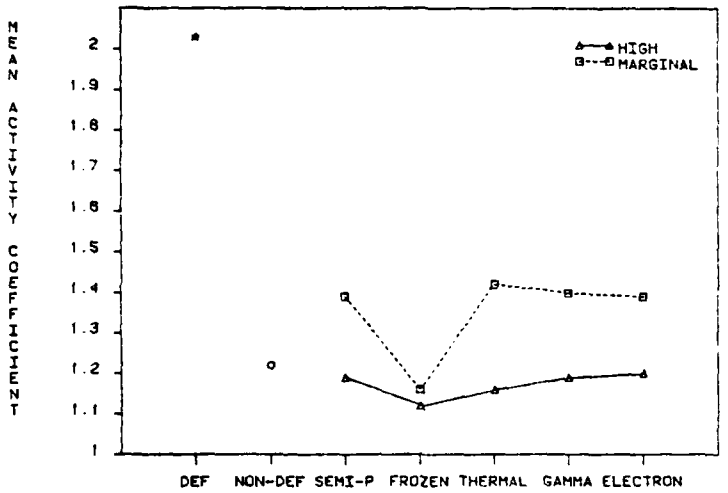

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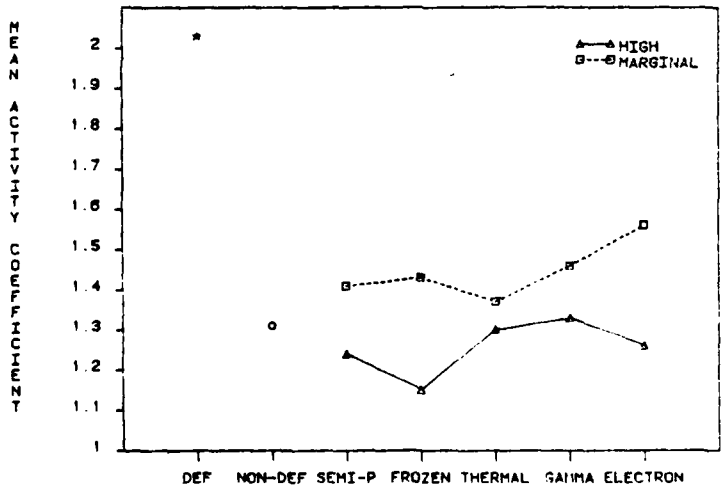
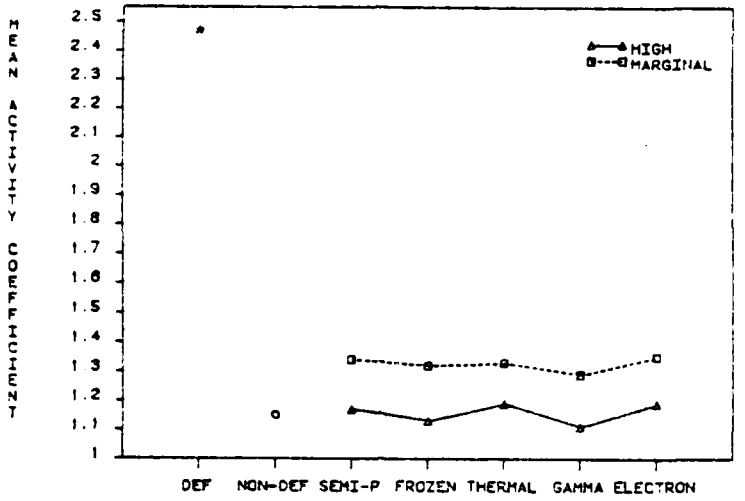


Day 28

Figure 19. Plasma alanine aminotransferase activity coefficients, males. Symbol at far left represents deficient group at Day 0.



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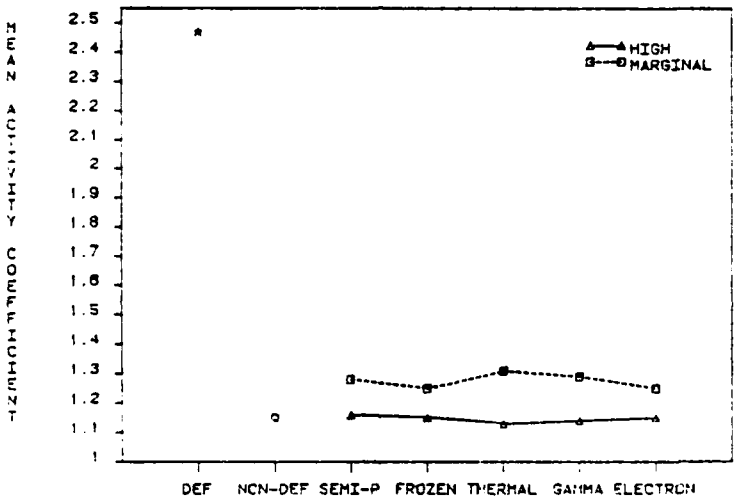

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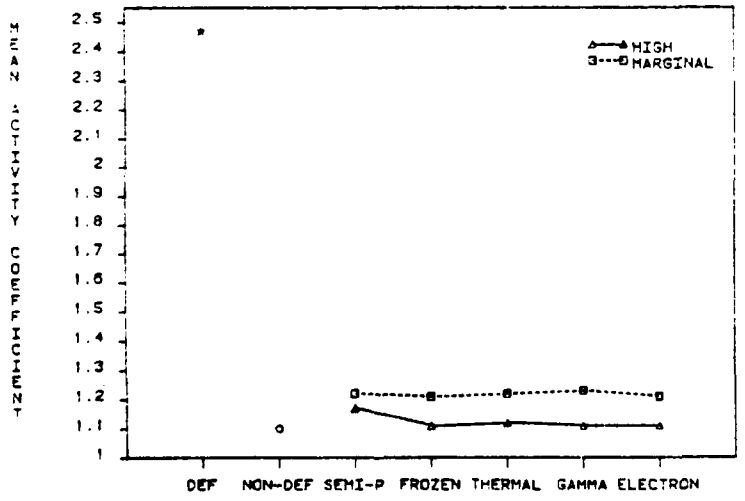
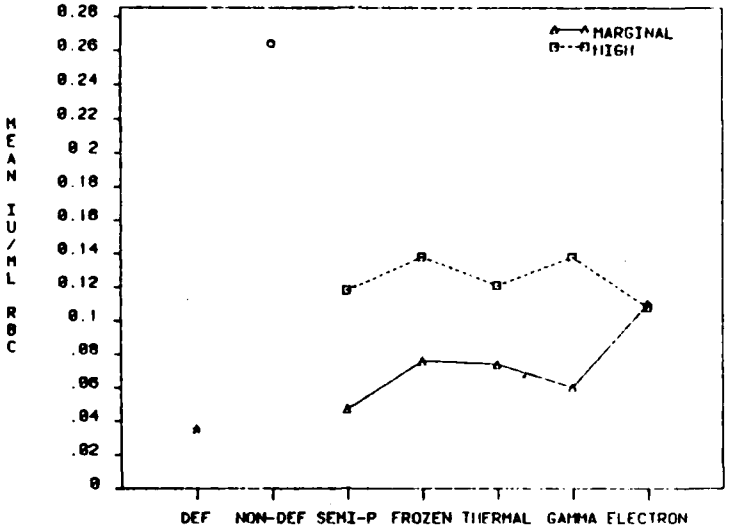


Day 28

Figure 20. Plasma alanine aminotransferase activity coefficients, females. Symbol at far left represents deficient group at Day 0.



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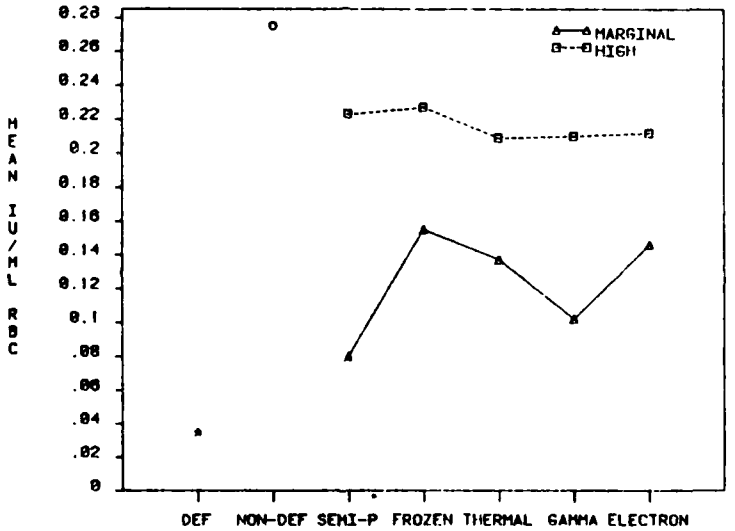

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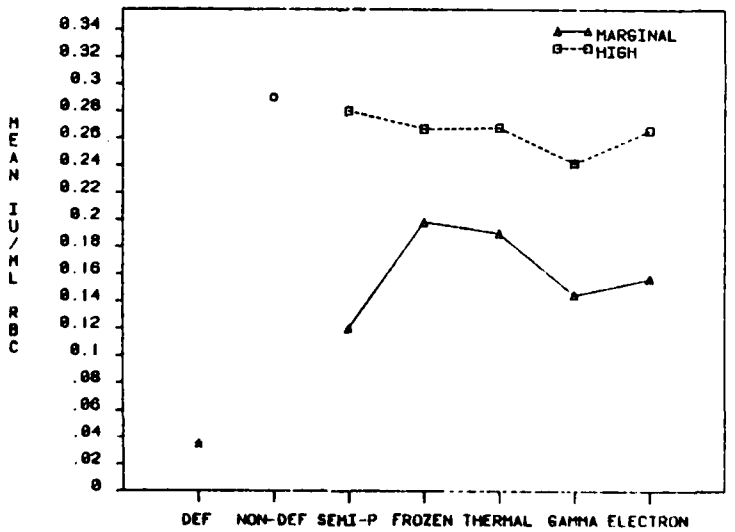
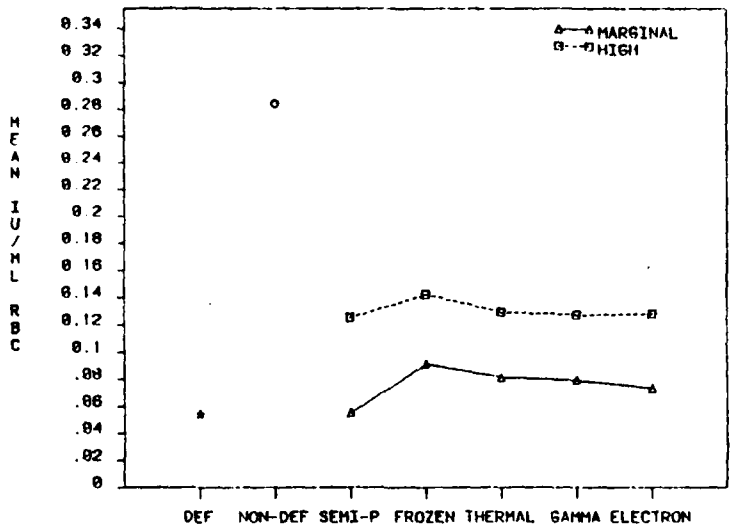
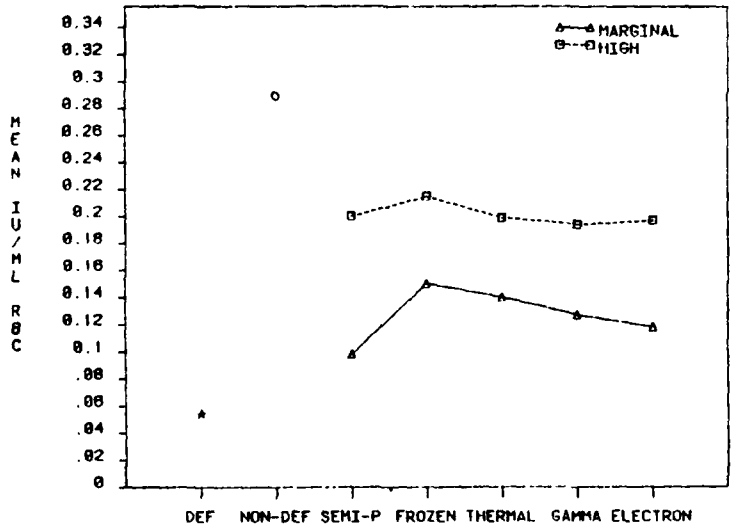


Day 28

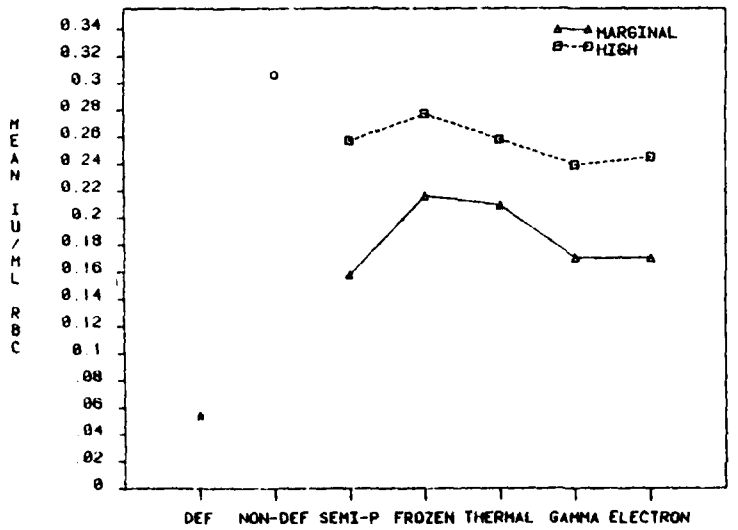
Figure 21. Erythrocyte alanine aminotransferase activity, males.



Day 7



Day 14



Day 28

Figure 22. Erythrocyte alanine aminotransferase activity, females.

LIST OF TABLES

APPENDIX B

Table 1. Vitamin B-6 contents of chicken test meats and repletion diets

	Test Meat or Repletion Diet				
	Semi-Purified	Frozen	Thermal	Gamma Irradiated	Electron Irradiated
Vitamin B-6 in Meat[1]					
mg/kg, wet wt. (n = 6)	-	2.4 ± 0.2	2.0 ± 0.2	1.2 ± .02	1.5 ± 0.2
Moisture Content %	-	64.5	64.0	63.4	65.2
Vitamin B-6 in Diets from Meat					
mg/kg, dry wt.	-	2.35	1.94	1.15	1.51
Pyridoxine·HCl Added to Diets					
mg/kg, dry wt.					
Marginal Diets	2.5	0.15	0.56	1.35	0.99
High B-6 Diets	12.0	9.65	10.06	10.85	10.49
Vitamin B-6 Assayed[1]					
mg/kg, wet wt.					
Marginal Diets (n = 7)	2.34 ± .26	1.79 ± .34	1.53 ± .28	1.53 ± .22	1.46 ± .27
High Diets (n = 9)	11.58 ± 1.09	8.31 ± .42	7.56 ± .50	7.90 ± .25	8.10 ± .38
Vitamin B-6 Content[2]					
mg/kg, dry wt.					
Marginal Diets	2.3	2.9	2.5	2.5	2.4
High Diets	11.6	13.5	12.3	12.3	13.4

[1]Mean ± S.D. [2]Calculated by estimating the moisture content of each diet based on moisture contents of meats. Moisture loss during grinding of meat and mixing with dry pre-mix was not taken into consideration.

Table 2. Schedule and diet codes for antivitamin B-6 studies[1]

Phase	Length of Phase	Diet Groups
1. (Quarantine)	1 week	A
2. (Depletion)	33-42 days	A,B
3. (Repletion)	4 weeks	A, C-L

Diet Code	Diet	Vitamin B-6 Level mg/kg dry weight
A	Semipurified	12.0 (non-deficient control group)
B	Semipurified	0 (deficient diet)
C	Semipurified	2.5
D	Semipurified	12.0
Chicken-Containing Diets		
E	Frozen Chicken	2.5
F	Frozen Chicken	12.0
G	Thermally Processed	2.5
H	Thermally Processed	12.0
I	Gamma Irradiated	2.5
J	Gamma Irradiated	12.0
K	Electron Irradiated	2.5
L	Electron Irradiated	12.0

[1]The experimental design was identical for both studies except one used all male animals and the other all females.

Table 3. Summary of quality control data for plasma and erythrocyte aminotransferase activities

	Within Day Precision			Between Day Precision		
	Mean	S.D.	C.V.	Mean	S.D.	C.V.
Plasma AST[1]						
Normal[2]	19.1	1.08	5.65	19.2	0.42	2.17
Normal[3]	23.4	1.17	5.00	23.4	0.88	3.73
Elevated[4]	42.1	1.87	4.44	42.1	1.31	3.11
Plasma AST Stimulated						
Normal[3]	36.4	1.39	3.82	36.4	0.69	1.88
Elevated[4]	68.9	2.07	3.00	69.1	1.53	1.21
Plasma ALT[1]						
Normal[2]	21.5	1.12	5.21	21.4	0.82	3.83
Normal[3]	30.5	1.07	3.51	30.4	1.23	4.05
Elevated[4]	86.2	2.89	3.35	85.9	3.21	3.73
Plasma ALT Stimulated						
Normal[3]	34.4	1.89	5.49	34.3	1.24	3.62
Elevated[4]	92.5	3.64	3.93	92.3	2.65	2.87
Erythrocyte AST[5]						
Normal, Male Rat	1.91	.050	2.62	1.90	.047	2.47
Deficient, Male Rat	0.50	.028	5.60	0.49	.017	3.46
Normal, Female Rat	1.86	.052	2.81	1.85	.059	3.19
Deficient, Female Rat	0.76	.041	5.46	0.75	.025	3.33
Erythrocyte AST, Stimulated						
Normal, Male Rat	2.09	.053	2.52	2.08	.083	3.99
Deficient, Male Rat	1.06	.044	4.20	1.05	.034	3.24
Normal, Female Rat	2.06	.060	2.90	2.05	.097	4.73
Deficient, Female Rat	1.32	.060	4.57	1.31	.071	5.42

[1]IU/liter. [2]Dade Monitrol I; American Hospital Supply, Miami, FL. [3]Sigma Enzyme Control -2N. [4]Sigma Enzyme Control -2E. [5]IU/ml packed red cells. Appropriate pools were prepared in advance, divided into aliquots and stored frozen at -70°C.

Table 1. Growth of pyridoxine-deficient rats repleted with semi-purified or chicken-based diets (males).

Group	Treatment	Initial Weight (g)[1]	Final Weight (g)[1] and (n)	Average Daily Gains (g) Week 1	Week 2	Week 3	Week 4	Overall Average (g)/Day
A	Non-deficient	368.4 ± 23.7	462.8 ± 35.6 (11)	4.1	3.0	1.2	4.9	3.3
C	Dry - 2.5	198.5 ± 20.6	379.6 ± 16.8 (9)	9.8	7.0	7.0	3.9	6.8
D	Dry - 12.0	198.4 ± 20.9	384.1 ± 36.9 (10)	11.1	7.2	6.0	4.0	6.9
E	Frozen - 2.5	198.6 ± 23.6	420.8 ± 29.4 (11)	13.7	8.4	6.9	4.6	8.2
F	Frozen - 12.0	196.0 ± 21.1	415.5 ± 24.7 (9)	14.3	8.3	6.5	4.9	8.3
G	Thermal - 2.5	198.2 ± 23.1	426.7 ± 27.2 (11)	14.7	8.7	7.7	4.3	8.6
H	Thermal - 12.0	198.2 ± 21.6	415.4 ± 12.6 (9)	13.5	7.5	6.4	4.0	7.6
I	Gamma - 2.5	199.2 ± 22.7	418.5 ± 34.2 (10)	14.0	7.8	7.8	3.6	8.1
J	Gamma - 12.0	197.5 ± 20.9	420.1 ± 32.2 (12)	14.2	8.9	6.4	4.3	8.2
K	Electron - 2.5	199.3 ± 19.8	437.2 ± 33.0 (11)	14.0	9.7	7.9	4.4	8.8
L	Electron - 12.0	196.6 ± 19.5	424.3 ± 32.2 (9)	14.0	9.4	6.7	5.3	8.7

[1]Mean ± S.D.

Table 5. Growth of pyridoxine-deficient rats repleted with semi-purified or chicken-based diets (females).

Group	Treatment	Initial Weight (g)[1]	Final Weight (g)[1] and (n)	Average Daily Gains (g)				Overall Average (g)/Day
				Week 1	Week 2	Week 3	Week 4	
A	Non-deficient	213.1 ± 14.8	273.4 ± 17.9 (11)	3.9	1.4	2.2	1.4	2.2
C	Dry - 2.5	153.1 ± 12.8	250.7 ± 25.0 (12)	6.2	3.2	2.5	1.9	3.5
D	Dry - 12.0	151.8 ± 13.3	254.9 ± 24.9 (12)	7.4	2.7	2.7	2.1	3.7
E	Frozen - 2.5	152.5 ± 13.0	279.6 ± 29.6 (11)	9.2	3.5	2.4	2.6	4.5
F	Frozen - 12.0	151.7 ± 12.9	273.8 ± 20.7 (11)	8.7	3.4	2.3	2.2	4.2
G	Thermal - 2.5	152.5 ± 13.4	274.1 ± 20.8 (10)	8.9	3.0	2.7	2.0	4.2
H	Thermal - 12.0	152.1 ± 13.4	273.6 ± 18.1 (10)	8.8	3.3	2.6	1.4	4.1
I	Gamma - 2.5	152.8 ± 12.4	274.6 ± 20.5 (12)	8.3	3.7	2.6	2.2	4.2
J	Gamma - 12.0	152.4 ± 13.6	271.7 ± 16.8 (11)	8.8	2.8	3.2	1.8	4.1
K	Electron - 2.5	152.5 ± 12.4	267.8 ± 20.5 (11)	8.8	3.3	1.5	2.8	4.2
L	Electron - 12.0	152.4 ± 13.8	269.3 ± 15.6 (10)	8.7	3.2	2.8	1.2	4.0

[1]Mean ± S.D.

Table 6. Analysis of variance (ANOVA) significance levels for erythrocyte aspartate aminotransferase activity.[1]

	Vitamin	Diet	Interaction
Erythrocyte Aspartate Aminotransferase Activity (Unstimulated)			
Males			
Day 7	.000	.000	.000
Day 14	.000	.139[2]	.035
Day 28	.000	.026[2]	.517
Females			
Day 7	.000	.001	.023
Day 14	.000	.080	.297
Day 28	.022	.527	.282
Erythrocyte Aspartate Aminotransferase Activity Coefficient			
Males			
Day 7	.000	.000[3]	.000
Day 14	.000	.000[3]	.000
Day 28	.000	.000	.000
Females			
Day 7	.000	.000	.000
Day 14	.000	.034	.107
Day 28	.000	.007	.004

[1]P values obtained by ANOVA on 5 food groups, 2 vitamin levels (Groups C through L). [2]Variances were significantly different by Bartlett's test. Kruskal-Wallis nonparametric analysis revealed no significant diet effect. [3]Diet differences were not significant ($P < .05$) by Newman-Keuls test. Examination of 95% confidence intervals did reveal apparent differences.

Table 7. Summary of erythrocyte aspartate aminotransferase activity in pyridoxine-deficient rats during repletion with semi-purified or chicken-based diets[1,2]

Days of Repletion	Non-Deficient Controls	Semi-Purified	Frozen	Thermal	Gamma	Electron
			Males - 2.5 mg Pyridoxine/kg			
7	-	0.97 ± .04[d]	1.25 ± .04[b]	1.24 ± .04[b]	1.14 ± .03[c]	1.40 ± .03[a]
14	-	1.79 ± .08	2.05 ± .09	1.98 ± .05	1.78 ± .03	1.99 ± .07
28	-	1.96 ± .06	1.94 ± .05	1.96 ± .04	1.85 ± .03	1.85 ± .04
			Males - 12.0 mg Pyridoxine/kg			
7	1.66 ± .03	1.41 ± 04[b]	1.65 ± .07[a]	1.50 ± .04[ab]	1.53 ± .03[ab]	1.40 ± .04[b]
14	1.86 ± .05	2.15 ± .06	2.19 ± .12	2.05 ± .06	2.16 ± .05	2.08 ± .05
28	1.87 ± .06	2.15 ± .06	2.12 ± .07	2.02 ± .04	1.98 ± .03	2.06 ± .05
			Females - 2.5 mg Pyridoxine/kg			
7	-	1.19 ± .05[b]	1.48 ± .04[a]	1.46 ± .03[a]	1.37 ± .03[a]	1.39 ± .04[a]
14	-	1.75 ± .06	1.92 ± .04	1.93 ± .04	1.86 ± .05	1.78 ± .05
28	-	1.88 ± .05	1.93 ± .04	2.00 ± .03	1.93 ± .03	1.89 ± .04
			Females - 12.0 mg Pyridoxine/kg			
7	1.77 ± .04	1.63 ± .05	1.69 ± .05	1.66 ± .03	1.63 ± .04	1.62 ± .04
14	1.92 ± .06	2.05 ± .04	2.15 ± .10	1.99 ± .05	2.06 ± .06	2.00 ± .03
28	1.88 ± .07	2.04 ± .04	2.00 ± .06	1.98 ± .03	1.98 ± .04	1.94 ± .04

[1]IU/ml red blood cells; mean ± S.E.M. Groups with different superscripts are significantly ($P < .05$) different by the Newman-Keuls test. [2]Day 0: males - non-deficient = 1.94 ± .04, deficient = 0.52 ± .02; females - non-deficient = 1.88 ± .03, deficient = 0.73 ± .01.

NOTE: Superscripts refer to within-day (horizontal) comparisons only.

Table 8. Summary of erythrocyte aspartate aminotransferase activity coefficients in pyridoxine-deficient rats during repletion with semi-purified or chicken-based diets[1,2]

Days of Repletion	Non-Deficient Controls	Semi-Purified	Frozen	Thermal	Gamma	Electron
			Males - 2.5 mg Pyridoxine/kg			
7	-	1.45 ± .02^{e}	1.26 ± .01^{b}	1.29 ± .01^{c}	1.33 ± .01^{d}	1.20 ± .01^{a}
14	-	1.26 ± .01	1.18 ± .00	1.18 ± .00	1.21 ± .01	1.18 ± .01
28	-	1.19 ± .01^{c}	1.15 ± .01^{a}	1.16 ± .01^{a}	1.17 ± .00^{b}	1.16 ± .01^{a}
			Males - 12.0 mg Pyridoxine/kg			
7	1.12 ± .01	1.17 ± .01	1.16 ± .01	1.16 ± .01	1.16 ± .01	1.19 ± .01
14	1.15 ± .00	1.16 ± .00	1.16 ± .00	1.15 ± .00	1.16 ± .01	1.15 ± .00
28	1.13 ± .01	1.13 ± .00	1.12 ± .01	1.13 ± .00	1.13 ± .01	1.13 ± .00
			Females - 2.5 mg Pyridoxine/kg			
7	-	1.33 ± .01^{c}	1.19 ± .01^{a}	1.22 ± .01^{ab}	1.23 ± .01^{b}	1.22 ± .01^{ab}
14	-	1.18 ± .01^{b}	1.14 ± .01^{a}	1.14 ± .01^{a}	1.17 ± .01^{ab}	1.16 ± .01^{ab}
28	-	1.15 ± .00^{c}	1.12 ± .00^{a}	1.12 ± .01^{a}	1.14 ± .00^{bc}	1.13 ± .01^{ab}
			Females - 12.0 mg Pyridoxine/kg			
7	1.11 ± .01	1.14 ± .01	1.14 ± .01	1.13 ± .01	1.15 ± .01	1.14 ± .01
14	1.10 ± .01	1.11 ± .01	1.11 ± .01	1.11 ± .01	1.12 ± .01	1.12 ± .01
28	1.10 ± .01	1.11 ± .01	1.10 ± .00	1.11 ± .00	1.11 ± .00	1.11 ± .01

[1]Activity coefficient = $\frac{\text{stimulated activity}}{\text{unstimulated activity}}$; mean ± S.E.M. Groups with different superscripts are significantly ($P < .05$) different by the Newman-Keuls test. [2]Day 0: males - non-deficient = 1.06 ± .01, deficient = 1 94 ± .03; females - non-deficient = 1.06 ± .00, deficient = 1.68 ± .02.

NOTE: Superscripts refer to within-day (horizontal) comparisons only.

Table 9. Analysis of variance significance levels for plasma aspartate aminotransferase activity[1]

	Plasma Aspartate Aminotransferase Activity (Unstimulated)		
	Vitamin	Diet	Interaction
Males			
Day 7	.000	.001	.008
Day 14	.059	.148	.375
Day 28	.143	.765	.796
Females			
Day 7	.002	.067	.118
Day 14	.062	.044[2]	.180
Day 28	.349	.005	.036
	Plasma Aspartate Aminotransferase Activity Coefficient		
Males			
Day 7	.000	.000	.000
Day 14	.000	.018	.536
Day 28	.797	.794	.761
Females			
Day 7	.001	.222	.029
Day 14	.000	.000	.004
Day 28	.000	.011	.045

[1]P values obtained by ANOVA on 5 food groups, 2 vitamin levels (Groups C through L). [2]Diet differences were not significant ($P < .05$) by Newman-Keuls test. Examination of 95% confidence intervals revealed apparent differences.

Table 10. Summary of plasma aspartate aminotransferase activity in pyridoxine-deficient rats during repletion with semi-purified or chicken-based diets[1,2]

Days of Repletion	Non-Deficient Controls	Semi-Purified	Frozen	Thermal	Gamma	Electron
			Males - 2.5 mg Pyridoxine/kg			
7	-	33.8 ± 0.9[b]	38.1 ± 1.3[a]	34.7 ± 0.8[b]	29.7 ± 1.2[c]	34.9 ± 1.0[ab]
14	-	45.9 ± 5.0	43.7 ± 1.4	44.3 ± 2.7	35.4 ± 2.0	44.7 ± 5.0
28	-	46.0 ± 2.7	45.1 ± 2.4	42.0 ± 5.2	37.2 ± 1.7	47.0 ± 6.3
			Males - 12.0 mg Pyridoxine/kg			
7	40.8 ± 1.7	42.9 ± 1.8	40.5 ± 1.2	43.5 ± 0.8	40.1 ± 0.9	41.6 ± 1.4
14	44.1 ± 1.5	51.9 ± 3.4	44.8 ± 1.5	47.1 ± 2.6	46.1 ± 3.4	43.4 ± 1.4
28	45.3 ± 2.1	45.0 ± 4.6	52.2 ± 9.6	48.0 ± 3.5	47.7 ± 5.0	47.9 ± 5.1
			Females - 2.5 mg Pyridoxine/kg			
7	-	42.0 ± 2.0	43.6 ± 3.1	34.9 ± 1.8	34.8 ± 2.3	36.4 ± 1.4
14	-	46.0 ± 2.7	51.0 ± 3.3	44.5 ± 2.9	38.0 ± 1.2	39.6 ± 3.3
28	-	52.1 ± 4.4[ab]	45.1 ± 2.5[ab]	58.0 ± 7.9[a]	41.6 ± 2.0[b]	44.8 ± 3.7[ab]
			Females - 12.0 mg Pyridoxine/kg			
7	53.2 ± 6.8	44.0 ± 1.6	43.5 ± 2.5	42.4 ± 2.7	46.1 ± 3.6	39.5 ± 1.5
14	47.8 ± 3.6	51.7 ± 4.9	46.1 ± 2.0	48.8 ± 3.4	41.3 ± 1.1	51.3 ± 5.9
28	51.2 ± 4.0	64.6 ± 6.5[a]	49.5 ± 2.0[b]	44.7 ± 1.4[b]	47.4 ± 2.4[b]	47.6 ± 2.3[b]

[1]IU/liter plasma; mean ± S.E.M. Groups with different superscripts are significantly ($P < .05$) different by the Newman-Keuls test. [2]Day 0: males - non-deficient = 45.4 ± 2.0, deficient = 17.3 ± 0.7; females - non-deficient = 43.2 ± 1.3, deficient = 15.2 ± 0.4.

NOTE: Superscripts refer to within-day (horizontal) comparisons only.

Table 11. Summary of plasma aspartate aminotransferase activity coefficients in pyridoxine-deficient rats during repletion with semi-purified or chicken-based diets[1,2]

Days of Repletion	Non-Deficient Controls	Semi-Purified	Frozen	Thermal	Gamma	Electron
			Males - 2.5 mg Pyridoxine/kg			
7	-	$1.55 \pm .03^{c}$	$1.38 \pm .01^{a}$	$1.47 \pm .01^{b}$	$1.55 \pm .02^{c}$	$1.42 \pm .01^{a}$
14	-	$1.37 \pm .02^{a}$	$1.41 \pm .06^{ab}$	$1.34 \pm .02^{b}$	$1.48 \pm .02^{b}$	$1.40 \pm .02^{ab}$
28	-	$1.34 \pm .03$	$1.35 \pm .02$	$1.36 \pm .03$	$1.29 \pm .05$	$1.33 \pm .05$
			Males - 12.0 mg Pyridoxine/kg			
7	$1.33 \pm .02$	$1.32 \pm .01$	$1.32 \pm .02$	$1.31 \pm .01$	$1.34 \pm .02$	$1.29 \pm .02$
14	$1.32 \pm .02$	$1.26 \pm .01$	$1.26 \pm .02$	$1.27 \pm .02$	$1.32 \pm .03$	$1.32 \pm .04$
28	$1.28 \pm .03$	$1.35 \pm .04$	$1.38 \pm .06$	$1.30 \pm .03$	$1.34 \pm .05$	$1.34 \pm .05$
			Females - 2.5 mg Pyridoxine/kg			
7	-	$1.38 \pm .02$	$1.26 \pm .03$	$1.41 \pm .04$	$1.39 \pm .03$	$1.32 \pm .04$
14	-	$1.38 \pm .01^{b}$	$1.32 \pm .01^{a}$	$1.38 \pm .02^{b}$	$1.44 \pm .01^{c}$	$1.42 \pm .02^{bc}$
28	-	$1.30 \pm .02^{a}$	$1.30 \pm .02^{a}$	$1.29 \pm .02^{a}$	$1.36 \pm .01^{b}$	$1.31 \pm .02^{ab}$
			Females - 12.0 mg Pyridoxine/kg			
7	$1.20 \pm .03$	$1.31 \pm .05$	$1.28 \pm .02$	$1.25 \pm .03$	$1.25 \pm .02$	$1.31 \pm .04$
14	$1.33 \pm .02$	$1.31 \pm .01$	$1.32 \pm .01$	$1.30 \pm .01$	$1.33 \pm .01$	$1.33 \pm .02$
28	$1.27 \pm .02$	$1.24 \pm .01$	$1.29 \pm .01$	$1.24 \pm .01$	$1.26 \pm .01$	$1.27 \pm .01$

[1]Activity coefficient = $\frac{\text{stimulated activity}}{\text{unstimulated activity}}$; mean ± S.E.M. Groups with different superscripts are significantly ($P < .05$) different by the Newman-Keuls test. [2]Day 0: males - non-deficient = 1.23 ± .01, deficient = 2.52 ± .06; females - non-deficient = 1.32 ± .02, deficient = 2.68 ± .05.

NOTE: Superscripts refer to within-day (horizontal) comparisons only.

Table 12. Analysis of variance significance levels for plasma alanine aminotransferase activity[1]

	Plasma Alanine Aminotransferase Activity (Unstimulated)		
	Vitamin	Diet	Interaction
Males			
Day 7	.000	.000	.679
Day 14	.000	.007	.558
Day 28	.000	.026[2]	.678
Females			
Day 7	.000	.005	.252
Day 14	.000	.012[2]	.890
Day 28	.000	.046[3]	.412
	Plasma Aminotransferase Activity Coefficient		
Males			
Day 7	.000	.000	.024
Day 14	.000	.004	.179
Day 28	.000	.407	.409
Females			
Day 7	.000	.030[2]	.915
Day 14	.000	.688	.323
Day 28	.000	.501	.690

[1]P values obtained by ANOVA on 5 food groups, 2 vitamin levels (Groups C through L). [2]Variances were significantly different by Bartlett's test. Kruskal-Wallis nonparametric analysis revealed significant diet effects at high vitamin level only. [3]Diet differences were not significant ($P < .05$) by Newman-Keuls test. Examination of 95% confidence intervals did reveal apparent differences.

Table 13. Summary of plasma alanine aminotransferase activity in pyridoxine-deficient rats during repletion with semi-purified or chicken-based diets[1,2]

Days of Repletion	Non-Deficient Controls	Semi-Purified	Frozen	Thermal	Gamma	Electron
			Males - 2.5 mg Pyridoxine/kg			
7	-	14.1 ± 0.8^{ab}	17.6 ± 0.9^{a}	17.5 ± 0.9^{a}	11.7 ± 0.8^{b}	15.5 ± 0.8^{ab}
14	-	14.5 ± 0.9^{ab}	17.9 ± 0.9^{a}	16.7 ± 1.0^{ab}	13.7 ± 0.5^{b}	14.0 ± 0.8^{ab}
28	-	13.4 ± 0.9	16.2 ± 1.1	16.5 ± 0.9	13.2 ± 0.8	14.5 ± 1.7
			Males - 12.0 mg Pyridoxine/kg			
7	17.6 ± 1.0	24.1 ± 1.1	24.7 ± 1.0	26.1 ± 1.2	21.8 ± 1.4	24.6 ± 2.0
14	19.1 ± 0.8	22.6 ± 1.0	22.6 ± 1.3	24.1 ± 1.4	20.9 ± 1.3	21.4 ± 1.0
28	18.1 ± 0.9	18.7 ± 0.9	19.1 ± 1.3	23.1 ± 2.9	17.9 ± 1.4	21.1 ± 0.7
			Females - 2.5 mg Pyridoxine/kg			
7	-	19.3 ± 0.9^{ab}	21.5 ± 1.4^{a}	17.1 ± 1.0^{b}	16.4 ± 0.8^{b}	17.2 ± 0.9^{b}
14	-	20.9 ± 1.0	22.5 ± 1.1	18.9 ± 0.8	18.3 ± 0.8	19.0 ± 0.7
28	-	21.2 ± 1.2	21.3 ± 1.2	20.1 ± 0.9	17.3 ± 0.8	16.9 ± 1.2
			Females - 12.0 mg Pyridoxine/kg			
7	22.2 ± 1.2	28.3 ± 1.4	26.1 ± 1.0	24.6 ± 1.2	25.4 ± 1.1	24.8 ± 1.2
14	24.6 ± 1.1	28.1 ± 1.0	28.9 ± 2.5	27.7 ± 1.0	25.2 ± 1.2	26.2 ± 0.6
28	23.7 ± 1.2	26.3 ± 2.0	24.6 ± 1.4	25.1 ± 0.9	24.8 ± 1.3	24.0 ± 0.7

[1]IU/liter plasma; mean ± S.E.M. Groups with different superscripts are significantly ($P < .05$) different by the Newman-Keuls test. [2]Day 0: males - non-deficient = 19.6 ± 1.4, deficient = 5.4 ± 0.3; females - non-deficient = 21.0 ± 0.8, deficient = 2.7 ± 0.1

NOTE: Superscripts refer to within-day (horizontal) comparisons only.

Table 14. Summary of plasma alanine aminotransferase activity coefficients in pyridoxine-deficient rats during repletion with semi-purified or chicken-based diets[1,2]

Days of Repletion	Non-Deficient Controls	Semi-Purified	Frozen	Thermal	Gamma	Electron
			Males - 2.5 mg Pyridoxine/kg			
7	-	1.59 ± .05[b]	1.34 ± .04[a]	1.39 ± .04[a]	1.55 ± .05[b]	1.43 ± .05[a]
14	-	1.39 ± .05[b]	1.16 ± .05[a]	1.42 ± .07[b]	1.40 ± .05[b]	1.39 ± .04[b]
28	-	1.41 ± .06	1.43 ± .08	1.37 ± .08	1.46 ± .11	1.56 ± .08
			Males - 12.0 mg Pyridoxine/kg			
7	1.30 ± .05	1.24 ± .01	1.21 ± .02	1.17 ± .02	1.25 ± .03	1.21 ± .03
14	1.22 ± .04	1.19 ± .04	1.12 ± .04	1.16 ± .03	1.19 ± .04	1.20 ± .05
28	1.31 ± .05	1.24 ± .05	1.15 ± .05	1.30 ± .04	1.33 ± .03	1.26 ± .06
			Females - 2.5 mg Pyridoxine/kg			
7	-	1.34 ± .02	1.32 ± .03	1.33 ± .03	1.29 ± .03	1.35 ± .03
14	-	1.28 ± .02	1.25 ± .03	1.31 ± .02	1.29 ± .02	1.25 ± .01
28	-	1.22 ± .02	1.21 ± .02	1.22 ± .02	1.23 ± .03	1.21 ± .02
			Females - 12.0 mg Pyridoxine/kg			
7	1.15 ± .03	1.17 ± .02	1.13 ± .02	1.19 ± .02	1.11 ± .02	1.19 ± .03
14	1.15 ± .02	1.16 ± .01	1.15 ± .02	1.13 ± .02	1.14 ± .02	1.15 ± .02
28	1.10 ± .02	1.17 ± .04	1.11 ± .02	1.12 ± .02	1.11 ± .02	1.11 ± .02

[1]Activity coefficients = $\frac{\text{stimulated activity}}{\text{unstimulated activity}}$; mean ± S.E.M. Groups with different superscripts are significantly ($P < .05$) different by the Newman-Keuls test. [2]Day 0: males - non-deficient = 1.20 ± .18, deficient = 2.03 ± .04; females - non-deficient = 1.17 ± .01, deficient = 2.47 ± .14.
NOTE: Superscripts refer to within-day (horizontal) comparisons only.

Table 15. Analysis of variance significance levels for erythrocyte alanine aminotransferase activity[1]

		Erythrocyte Alanine Aminotransferase Activity (Unstimulated)		
		Vitamin	Diet	Interaction
Males				
	Day 7	.000	.000	.000
	Day 14	.000	.000	.000
	Day 28	.000	.000	.000
Females				
	Day 7	.000	.000	.067
	Day 14	.000	.000	.003
	Day 28	.000	.000	.025
		Erythrocyte Alanine Aminotransferase Activity (Coefficient)		
Males				
	Day 7	.000	.004	.000
	Day 14	.000	.021	.012
	Day 28	.000	.004	.054
Females				
	Day 7	.000	.283	.362
	Day 14	.000	.397	.540
	Day 28	.600	.429	.867

[1]P values obtained by ANOVA on 5 food groups, 2 vitamin levels (Groups C through L).

Table 16. Summary of erythrocyte alanine aminotransferase activity in pyridoxine-deficient rats during repletion with semi-purified or chicken-based diets[1,2]

Days of Repletion	Non-Deficient Controls	Semi-Purified	Frozen	Thermal	Gamma	Electron
			Males - 2.5 mg Pyridoxine/kg			
7	-	.047 ± .002^{c}	.076 ± .003^{b}	.074 ± .002^{b}	.060 ± .002^{bc}	.110 ± .003^{a}
14	-	.080 ± .004^{c}	.155 ± .005^{a}	.137 ± .006^{a}	.102 ± .005^{b}	.146 ± .005^{a}
28	-	.120 ± .008^{c}	.198 ± .007^{a}	.190 ± .008^{a}	.145 ± .009^{b}	.157 ± .007^{b}
			Males - 12.0 mg Pyridoxine/kg			
7	.264 ± .009	.118 ± .005^{b}	.138 ± .006^{a}	.121 ± .004^{ab}	.138 ± .014^{a}	.108 ± .005^{b}
14	.275 ± .008	.223 ± .007$_{a}$	.227 ± .008$_{a}$	.209 ± .010$_{a}$	.210 ± .005$_{b}$	.212 ± .006$_{a}$
28	.290 ± .011	.280 ± .009^{a}	.267 ± .006^{a}	.268 ± .010^{a}	.242 ± .007^{b}	.266 ± .010^{a}
			Females - 2.5 mg Pyridoxine/kg			
7	-	.055 ± .002^{c}	.091 ± .002^{a}	.081 ± .002^{ab}	.079 ± .003^{ab}	.073 ± .003^{b}
14	-	.098 ± .000^{d}	.150 ± .004^{a}	.140 ± .005^{ab}	.127 ± .004^{bc}	.118 ± .006^{c}
28	-	.158 ± .006^{b}	.216 ± .007^{a}	.209 ± .007^{a}	.170 ± .006^{b}	.170 ± .008^{b}
			Females - 12.0 mg Pyridoxine/kg			
7	.284 ± .006	.125 ± .004^{b}	.142 ± .007^{a}	.129 ± .006^{b}	.127 ± .004^{b}	.128 ± .006^{b}
14	.289 ± .008	.200 ± .006$_{ab}$	.215 ± .007$_{a}$	.199 ± .007$_{ab}$	.194 ± .006$_{b}$	.197 ± .008$_{b}$
28	.306 ± .009	.257 ± .006^{ab}	.277 ± .010^{a}	.258 ± .010^{ab}	.239 ± .009^{b}	.245 ± .008^{b}

[1]IU/ml red blood cells; mean ± S.E.M. Groups with different superscripts are significantly ($P < .05$) different by the Newman-Keuls test. [2]Day 0: males - non-deficient = 0.283 ± .007, deficient = .035 ± .001; females - non-deficient = .268 ± .008; deficient = .054 ± .002.

NOTE: Superscripts refer to within-day (horizontal) comparisons only.

Table 17. Summary of erythrocyte alanine aminotransferase activity coefficients in pyridoxine-deficient rats during repletion with semi-purified or chicken-based diets[1,2]

Days of Repletion	Non-Deficient Controls	Semi-Purified	Frozen	Thermal	Gamma	Electron
			Males - 2.5 mg Pyridoxine/kg			
7	-	1.18 ± .02$_c$	1.10 ± .01$_a$	1.12 ± .01	1.16 ± .01	1.09 ± .01
14	-	1.07 ± .01	1.04 ± .01	1.04 ± .01^{ab}	1.06 ± .01^{bc}	1.05 ± .01^{ab}
28	-	1.07 ± .01^b	1.04 ± .01^a	1.05 ± .01^a	1.05 ± .01^a	1.05 ± .01^a
			Males - 12.0 mg Pyridoxine/kg			
7	1.09 ± .04	1.07 ± .01	1.07 ± .01	1.08 ± .01	1.07 ± .01	1.08 ± .01
14	1.05 ± .01	1.04 ± .01	1.04 ± .01	1.03 ± .01	1.03 ± .01	1.04 ± .01
28	1.04 ± .01	1.04 ± .01	1.04 ± .01	1.03 ± .01	1.04 ± .01	1.04 ± .01
			Females - 2.5 mg Pyridoxine/kg			
7	-	1.12 ± .02	1.08 ± .01	1.12 ± .01	1.09 ± .01	1.10 ± .02
14	-	1.08 ± .01	1.08 ± .01	1.07 ± .01	1.07 ± .01	1.08 ± .01
28	-	1.04 ± .01	1.03 ± .01	1.03 ± .01	1.05 ± .01	1.03 ± .01
			Females - 12.0 mg Pyridoxine/kg			
7	1.05 ± .01	1.07 ± .01	1.06 ± .01	1.06 ± .01	1.06 ± .01	1.07 ± .01
14	1.06 ± .01	1.06 ± .01	1.06 ± .01	1.06 ± .01	1.05 ± .01	1.05 ± .01
28	1.03 ± .01	1.04 ± .01	1.03 ± .01	1.02 ± .01	1.03 ± .01	1.03 ± .01

[1]IU/ml red blood cells; mean ± S.E.M. Groups with different superscripts are significantly ($P < .05$) different by the Newman-Keuls test. [2]Day 0: males - non-deficient = 1.06 ± .01, deficient = 1.2 ± .01; females - non-deficient = 1.05 ± .01, deficient = 1.16 ± .02

NOTE: Superscripts refer to within-day (horizontal) comparisons only.

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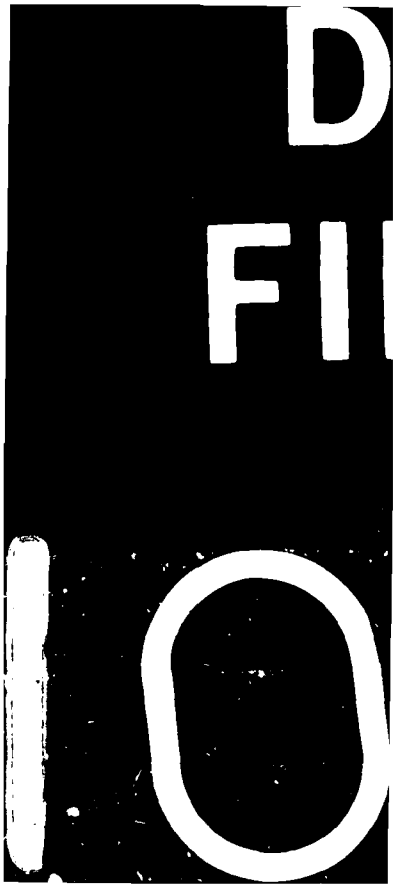
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